2.

Tagung der DGHM Fachgruppe

Mikrobiota, Probiota und Wirt
Microbiota, Probiota and Host

23.- 25. April 2009
Kultur und Bildungszentrum
Kloster Seeon / Chiemsee

Weitere Infos unter:
DGHM@wzw.tum.de
http://www.dghm.org/red/fachgruppen/

April 23, 2009

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology and the Organizing Committee, welcome to the 2nd 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. The gut acts as a highly selective barrier and communication organ between the environment and the intestinal immune system responsible for the regulation of metabolism and immunity in the host. It has been proposed to complement the search for disease susceptibility genes in the human genome with the analysis of the gut “microbiome”, considering the fact that health or disease is being determined by the complex interaction of the host with its gut microbial ecosystem. The peaceful and productive coexistence of the host with its gut microbiota is tightly controlled at various levels and a failure of this homeostasis is thought to contribute to the development of inflammation-driven chronic pathologies.
This DGHM section aims to integrate a panel of multidisciplinary experts relevant to novel aspects in microbiology and immunology. 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. This platform should enable cross-sectional discussions in the understanding for the role of bacteria-host interactions in the development or prevention of 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 Thursday, April 23

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

17^{15} - 18^{15}	Keynote Lecture: **R. B. Sartor**, University of North Carolina, Chapel Hill, North Carolina, USA ***Host-microbial interactions in the pathogenesis of IBD***

18^{15} - 18^{30}	DGHM Section Short Report

18^{45}	Dinner

LATE NIGHT SESSION

20^{00} – 22^{20} Chair: S. Meuer, Institute of Immunology, University Heidelberg

O. Pabst, Institute of Immunology, Hannover Medical School
The topography of the intestinal immune system

A. Diefenbach, Institute of Med. Microbiology & Hygiene, University of Freiburg
Commensal microflora is required for the differentiation of mucosal IL-22-producing cells

M. Hornef, Inst. for Medical Microbiology & Hospital Epidemiology, Hannover Medical School
Innate immune recognition and response by intestinal epithelial cells

J. Buer, Institute for Medical Microbiology, University Hospital Essen
The biological functions of new emerging CD4 T cells in intestinal inflammation

J. Wehkamp, Clinical Pharmakology, IKP Stuttgart
Epithelial stem cells and innate immune defenses in intestinal inflammation

C. Becker, Institute for Molecular Medicine, University Mainz
STAT3 as a central regulator of tissue homeostasis in infection and inflammation of the gut

D. Haller, Biofunctionality, Centre for Diet and Disease, TU München
ER and mitochondrial stress signaling under conditions of chronic intestinal inflammation

22^{30}	**Beer in the Bar** – An Invitation!

PROGRAM Friday, April 24

08^{30} - 09^{30} Keynote Lecture: **C. Kirschning**, Institute of Medical Microbiology, UK Essen
Therapeutic intervention in pattern recognition outside and TLR – inflammasome crosstalk within immune cells

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

MUCOSAL IMMUNOLOGY

10^{00} – 11^{30} Chair: S. C. Bischoff, Department of Nutritional Medicine, University Hohenheim

J. Beisner, Dr. Margarete Fischer-Bosch-Insitute of Clinical Pharmacology, Stuttgart
α-Defensin HD-5 and WNT signalling transcription factor TCF-4 in pediatric patients with Crohn´s Disease

A.M. Westendorf, Institute for Medical Microbiology, University Hospital Essen
Induction and expansion of regulatory T cells at mucosal surfaces

J.-S. Frick, Institute of Med. Microbiology & Hygiene, University of Tübingen
Commensal intestinal bacteria as potential inducers or inhibitors of $CD4^+$ T-cell induced colitis

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

J.H. Niess, Department of Internal Medicine I, University of Ulm
The commensal gut flora drives the accumulation of CX3CR1 dendritic cells responsible for the induction of Th1 and Th17 responses in the large intestine

A. Mortha, Institute for Med. Microbiology & Hygiene, University of Freiburg
Development of natural killer cell-like lymphoid tissue inducer cells is driven by the commensal microflora

11^{30} - 12^{00} Keynote Lecture: **B. Siegmund**, Gastroenterology, Department of Medicine, Charité Berlin
Metabolism and chronic intestinal inflammation: Signals from the fat

PROGRAM Friday, April 24

12^{00} - 14^{30} Lunch and Guided Tour through the Monastery

PROBIOTICS

14^{30} – 16^{15} Chair: W. Kruis, Internal Medicine, Evang.Krankenhaus Kalk

K. Fink, Institute of Med. Microbiology & Hygiene, University Tübingen
Increased mortality of E.coli Nissle 1917 mono-colonized RAG1 deficient mice upon $CD4^+$ T cell transfer

J. Preising, Institute for Microbiology and Biotechnology, University of Ulm
Anti-inflammatory activity of a B. bifidum strain in RAG1 $^{-/-}$ colitic mice

A. Sturm, Division of Hepatology & Gastroenterology, Charité Berlin
Escherichia coli Nissle 1917 induces apoptosis of peripheral $\gamma\delta$ T cells

C. Rasche, Department of Dermatology and Allergy, Charité Berlin
Modulation of the allergic response by inactivated non-pathogenic E. coli Nissle

T.A. Ölschläger, Institute for Molecular Infection Biology, University of Würzburg
The flagella of the probiotic Escherichia coli strain Nissle 1917 – a multipurpose tool

C. Pöhlmann, Institute of Med. Microbiology & Hygiene, TU Dresden
Secretion of biologically active recombinant IL-10 via a modified hemolysin transport system in Escherichia coli Nissle 1917

S. Hummel, Department of Infectiology, Centre for Molecular Biology of Inflammation (ZMBE), Münster
Gram-negative and gram-positive probiotic bacteria do it differently

16^{15} – 17^{30} Coffee Break / **Guided Poster Session**

17^{30} – 18^{30} Keynote Lecture: **M. Kalliomäki**, University of Turku, Finland
Probiotics in the prevention of atopic diseases

19^{30} Dinner

PROGRAM Saturday, April 25

08^{30} - 09^{30} Keynote Lecture: **J. Doré**, INRA, Jouy-en-Josas Cedex, France
Analysis of the human microbiome

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

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

MICROBES AND HOST

10^{00} – 11^{30} Chair: J.-S. Frick, Institute of Med. Microbiology & Hygiene, University Tübingen

J. Pott, Inst. for Medical Microbiology & Hospital Epidemiology, Hannover Medical School
Invasion-dependent recognition of Mycobacterium avium subsp. paratuberculosis

J. Vogel-Scheel, German Institute of Human Nutrition, Potsdam- Rehbrücke
Analysis of physiological status of Escherichia coli in different regions of the gastrointestinal tract in a gnotobiotic mouse model

R. Plickert, Institute of. Microbiology & Hygiene, Charité Berlin
MyD88 mediated TLR9 sensing modulated by TLR9 antagonist in GvHD

D. Mailänder-Sánchez, UK Tübingen
Impact of lactobacillus species on localised Candida albicans infection and the mucosal innate immune response

C.J. Chou, Nestlé Research Center, Lausanne, Switzerland
Gut microbiota modulation improves glucose tolerance of mice with insulin resistance

T. Werner, Biofunctionality, Centre for Diet and Disease, TU München
Reduction of dietary iron and systemic iron replenishment inhibit the development of chronic ileitis in TNF $^{\Delta ARE/WT}$ mice targeting endoplasmic reticulum stress mechanisms in intestinal epithelial cells (IEC)

11^{30} Lunch and Departure

PROGRAM
Thursday, April 23

HOST-MICROBIAL INTERACTIONS IN THE PATHOGENESIS OF IBD

R. B. Sartor

Midgette Distinguished Professor of Medicine, Microbiology and Immunology, University of North Carolina, Center for Gastrointestinal Biology and Disease, Chapel Hill, USA, rbs@med.unc.edu

We coexist with a huge load of complex enteric bacteria that outnumber our mammalian cells at least 10:1. These predominantly anaerobic bacteria are metabolically active and produce butyrate and other short chain fatty acids that are the primary fuel of colonic epithelial cells. These commensal bacteria produce adjuvants and antigens that stimulate innate and adaptive cells that mediate both regulatory and effector mucosal immune responses. We hypothesize that chronic relapsing Crohn's disease is due to overly aggressive T cell responses to a subset of functionally abnormal enteric commensal bacteria in susceptible hosts. Genetic susceptibility is determined by genes that encode either epithelial barrier function/healing, immune regulation or bacterial killing. Environmental triggers that transiently break the mucosal barrier or alter bacterial composition are responsible for the initiation or reactivation of inflammation. Four genes associated with Crohn's disease, including NOD2, affect bacterial killing. Polymorphisms of NOD2 are associated with defective ileal α defensin production and clearance of intracellular bacteria, possibly accounting for the ileal phenotype of this genotype. A developing theory of Crohn's disease is that defective innate immune clearance of bacteria leads to persistent antigenic induction of effector TH1 and TH17 cells that mediate inflammation. Gut bacteria are implicated in the pathogenesis of Crohn's disease through immune responses, the therapeutic benefit of antibiotics and probiotics, and increased presence of adherent-invasive *E. coli* strains in ileal Crohn's disease. We have demonstrated a necessary role of commensal enteric bacteria in chronic, immune- mediated experimental colitis in susceptible rodents with induction of bacterial antigen-specific TH1 and TH17 responses. These responses are host specific and bacterial species and strain specific, with functionally different *E. coli* strains having differential abilities to induce disease in monoassociated IL-10-/- mice, but no inflammation in wild type controls. These

colitogenic *E. coli* share the ability to adhere to, invade and translocate across colonic epithelial cells and to persist within and resist killing by macrophages. It is likely that an aggressive form of Crohn's disease may occur when a susceptible host with defective clearance of intracellular bacteria is colonized by opportunistic Commensal bacteria that are resistant to intracellular killing by macrophages. Ulcerative colitis is more difficult to understand, but may be primary epithelial injury caused by secretion of toxic metabolic products , such as hydrogen sulfide or reactive oxygen radicals, by luminal bacteria, defective degradation of reactive oxygen species, deficient energy metabolism or stress responses. This epithelial injury then leads to secondary increased uptake of bacterial antigens, which activate lamina propria T cell to further cause tissue injury. Finally, some IBD patients have evidence of altered bacterial composition (dysbiosis), which opens the possibility for therapeutic manipulation of the enteric microbiota.

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D. Haller, Biofunctionality, Centre for Diet and Disease, TU München
ER and mitochondrial stress signaling under conditions of chronic intestinal inflammation

THE TOPOGRAPHY OF THE INTESTINAL IMMUNE SYSTEM

Oliver Pabst

Hannover Medical School, Institute of Immunology, Carl-Neuberg Str.1 30625 Hannover, Germany; Pabst.Oliver@MH-Hannover.de

Scientific Interests:

Functional Anatomy of the Intestinal Immune System

1) *The role of the chemokine receptor CCR9 for immune cell migration into the small intestine*. We demonstrated that IgA-secreting plasma cells require CCR9 for entering the intestinal lamina propria. We characterized the function of CCR9 for a newly described population of intestinal plasmacytoid dendritic cells (DC) and we investigated the role of lamina propria DC in regulating CCR9 expression on activated gut seeking T cells. Currently, we focus on the capacity of lymphatic stroma cells that via production of retinoic acid contribute to the regulation of CCR9 on activated T cells.

2) *Function and organogenesis of "solitary intestinal lymphoid tissue".* Besides well recognized Peyer´s patches numerous smaller lymphoid structures have been described in the small intestine. These lymphoid aggregated have been thought to represent phenotypically and functionally distinct types of structures. We challenged this view and suggested that phenotypically different lymphoid aggregates merely represent particular manifestations of a common dynamic lymphoid structure that we termed as "solitary intestinal lymphoid tissue" (SILT). This new concept now meets acceptance in the scientific community as exemplified by numerous citations in other reports. We reported that SILT provides a productive port of entry for *Salmonella* leading to considerable intestinal inflammation in these structures in *Salmonella* infected mice and adapts in response to microbial stimulation. We now focus at understanding how the process of microbial driven adaptation is regulated.

3) *Mesenteric lymph nodes at the centre of intestinal tolerance.* Combining microsurgery and genetic approaches we explored the function of DC in oral tolerance induction. We reported that oral tolerance induction essentially requires

the chemokine receptor CCR7-dependent cell bound antigen transport from the intestine into the draining mesenteric lymph nodes. We now concentrate on the mechanisms leading to Oral tolerance on a cellular basis. In particular we try to understand how regulatory T cells are induced in the intestinal immune system.

Modelsystems:

Infection models (S. Typhimurium, Trichuris muris), germ-free mice, transgenic mouse strains (CCR7, CCR9, CXCR5, CX3CR1, OT-I, OT-II, FoxP3-GFP and other), microsurgery (adenectomy and lymph node transplantation, intra lymphatic injection, ligated intestinal loop, intra vital two photon microscopy)

The most impressive visible sign of immunological activity in the gut are Peyer´s patches, that are easily spotted already by the naked eye as aggregated follicles bulging out of the intestinal tube. However, besides Peyer´s patches, solitary intestinal lymphoid tissue (SILT) provides a structural platform to efficiently initiate immune responses in the murine small intestine. SILT consists of dynamic lymphoid aggregates that are heterogeneous in size and composition, ranging from small clusters of mostly lineage-negative cells known as cryptopatches to larger isolated lymphoid follicles rich in B cells. Here we report a novel technique that allows monitoring the dynamic behaviour of individual SILT over time. We demonstrate that colonization of germ free mice with commensal bacteria provokes an adjustment of the spectrum of SILT to that observed under specific pathogen free conditions by the conversion of pre-existing lymphoid structures into larger-sized SILT. Further enhanced microbial stimulation by means of oral infection with the enteropathogen *Salmonella* yields SILT that exceed the size spectrum of structures observed under pathogen free conditions. *Salmonella* directly infects SILT triggering a vigorous inflammatory response and immunopathology that leads to enlargement and morphological destruction of SILT. Dissemination of Salmonella from infected small intestinal tissue into the periphery depends on dendritc cell migration. Dendritic cells constitutively traffic from the intestine to the gut draining mesenteric lymph nodes (MLN) in a chemokine receptor CCR7-dependent mechanism that is essential to induce tolerance to food proteins. Salmonella exploit this pathway and utilize migrating dendritic cells to reach the MLN. However, under normal conditions the MLN prevent spreading of intestinal dendritic cells and thereby *Salmonella* beyond these lymph nodes. Surgical removal of the MLN results in increased numbers of *Salmonella* reaching systemic sites early after infection, thereby rendering otherwise resistant mice susceptible to fatal systemic disease development. This suggests that the MLN provide a vital barrier, shielding systemic compartments from DC-mediated dissemination of *Salmonella*. Thus confinement of *Salmonella* in gut associated lymphoid tissue and MLN delays massive extra-intestinal dissemination and at the same time allows for the establishment of protective adaptive immune responses.

COMMENSAL MICROFLORA IS REQUIRED FOR THE DIFFERENTIATION OF MUCOSAL IL-22-PRODUCING CELLS

Stephanie Sanos[1], Arthur Mortha[1], Viet L. Bui[1], Elina Kivi[1], Caroline Johner[2], and Andreas Diefenbach[1]

[1] IMMH, Institute of Medical Microbiology & Hygiene, University of Freiburg, Medical Center, Hermann-Herder-Strasse 11, 79104 Freiburg;
[2] MPI-IB, Max-Planck-Institute of Immunobiology, Stübeweg 51, 79108 Freiburg, Germany; andreas.diefenbach@uniklinik-freiburg.de

The mucosal immune system of the intestine is separated by a single layer of epithelial cells from a vast array of microbes. Cues from the commensal microflora are needed to maintain epithelial homeostasis, but the molecular and cellular identities of these cues are unclear. We show that signals from the commensal microflora contribute to the differentiation of a lymphocyte population co-expressing stimulatory NK cell receptors and the transcription factor RORγt that constitutively produce interleukin (IL)-22. Emergence of these IL-22-producing RORγt^{hi} NKp46^{+} cells depended on the expression of RORγt but not on IL-15, indicating that these cells may not be NK cells but rather be derived from lymphoid tissue-inducer (LTi) cells. Interestingly, these RORγt^{hi} NKp46^{+} cells are located together with RORγt^{hi} NKp46^{-} LTi cells within the cryptopatches of the small intestine and both cell populations constitutively produced IL-22. IL-22 regulated epithelial expression of proteins involved in antimicrobial protection of epithelial surfaces and tissue regeneration (e.g., RegIII proteins). Mice genetically lacking RORγt do not develop cryptopatches and epithelial expression of RegIII proteins in the crypts was strongly diminished. We propose that IL-22 released by cryptopatch-resident cells promotes mucosal homeostasis.

INNATE IMMUNE RECOGNITION AND RESPONSE BY INTESTINAL EPITHELIAL CELLS

Mathias Hornef

Institute for Medical Microbiology and Hospital Epidemiology, Hanover Medical School, Hanover, Germany, hornef.mathias@mh-hannover.de

The innate immune system distinguishes between self and the presence of microbial organisms by the recognition of specific microbial structures so-called pathogen-associated molecuar patterns (PAMPs). Members of the family of toll-like receptors (TLR) mediate cellular stimulation and the secretion of proinflammatory mediators upon exposure to highly conserved and functionally essential microbial structures such as cell wall constituents or nucleic acids. TLR expression was first detected on cells of the myeloid lineage such as DCs and macrophages but recent work has indicated the presence of at least a subset of functionally intact TLRs also on intestinal epithelial cells. Mucosal TLR expression might thereby contribute to the local host defence in the event of microbial challenge e.g. by the production of antimicrobial peptides. However, the intestinal mucosa is permanently colonized by a complex and very dynamic microbial flora and exposed to microbial constituents through the oral uptake of nutrients and water. Yet mechanisms that distinguish between infection and physiological colonization and prevent inappropriate TLR activation and cellular stimulation are largely undefined. We analyzed TLR4 expression and ligand-mediated cell activation of intestinal epithelial cells. We investigated negative regulatory mechanisms of epithelial TLR4 signalling during postnatal development. Our results demonstrate acquisition of a TLR tolerant state during a transient epithelial cell activation shortly after birth. They also suggest significant changes in the repertoire of antimicrobial peptide production during the postnatal period. Both mechanisms might significantly contribute to facilitate bacterial colonization and the establishment of gut homeostasis.

THE BIOLOGICAL FUNCTIONS OF NEW EMERGING CD4 T CELLS IN INTESTINAL INFLAMMATION

Jan Buer

Institute for Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Germany, buer.jan@uk-essen.de

The intestinal mucosa is continuously exposed to both potential pathogens and beneficial commensal microorganisms. This leads to constant immunological stimulation creating a requirement for a regulatory network governing the balance between tolerance and immunity. Mucosal T cells must remain immunologically hyporesponsive to commensal bacteria while retaining their capacity to respond to a pathogenic challenge. Uncontrolled and persistent effector T cell responses can drive the onset of severe intestinal inflammation. Here we will discuss the Villin-HA mouse model as a new platform to study the biological impact of new emerging CD4 T cell subsets in maintaining or impairing mucosal homeostasis in the gut.

EPITHELIAL STEM CELLS AND INNATE IMMUNE DEFENSES IN INTESTINAL INFLAMMATION

Jan Wehkamp

Clinical Pharmacology, IKP Stuttgart, Auerbachstr. 112, 70376 Stuttgart, jan.wehkamp@ikp-stuttgart.de

Defensins are endogenous antibiotics with broad microbicidal activity. A disturbed antimicrobial defense, as provided by Paneth and other epithelial defensins, seems to be a critical factor in the pathogenesis of inflammatory bowel diseases. Conspicuously, there is a relative lack of Paneth-cell alpha-defensins in ileal Crohn's disease (CD), both in the absence of a pattern recognition receptor nucleotide-binding oligomerization domain 2 (NOD2) frameshift mutation and, even more pronounced, in its presence. This deficit is independent of concurrent active inflammation and cannot be seen in active small intestinal ulcerative colitis (UC; pouchitis) as well as NOD2 wild-type graft vs. host ileitis. After intestinal transplantation, in case of NOD2 mutation, defensins are decreased before the onset of inflammation. In the majority of patients, the Paneth-cell deficiency is mediated by Wnt-TCF4, which suggests a disturbed Paneth-cell differentiation. In this talk, different other mechanisms involved in intestinal stem cell regulation and defects in innate immunity will be discussed and outlined. In both ileal and colonic CD, the lack in defensins results in a broadly diminished antibacterial killing by the mucosa, which can also be found independent of inflammation. In summary, the main disease locations can be linked to distinct mechanisms of epithelial barrier dysfunction. We believe that these data will provide the ground for new therapeutic avenues which will aim to strengthen the innate immune system at the mucosal-bacterial interface.

STAT3 AS A CENTRAL REGULATOR OF TISSUE HOMEOSTASIS IN INFECTION AND INFLAMMATION OF THE GUT

Christoph Becker

Institut für Molekulare Medizin, Universität Mainz, Langenbeckstr. 1, 55131 Mainz, chbecker@uni-mainz.de

STAT3 is a pleiotropic transcription factor with important functions in cytokine signalling in a variety of tissues. However, the role of STAT3 in the intestinal epithelium is not well understood. Development of colonic inflammation in Crohn´s disease in humans and experimental DSS colitis in mice leads to a rapid induction of STAT3 activity in intestinal epithelial cells (IEC). Studies in genetically engineered mice showed that epithelial STAT3 activation in DSS colitis is dependent on IL-22 rather than IL-6. IL-22 was secreted by colonic CD11c+ cells in response to Toll-like receptor stimulation. Conditional knockout mice with an IEC specific deletion of STAT3 activity were highly susceptible to experimental models of gut infection and inflammation, indicating that epithelial STAT3 regulates gut homeostasis. $STAT3^{IEC\text{-}KO}$ mice, upon induction of colitis, showed a striking defect of epithelial restitution. Gene chip analysis indicated that STAT3 regulates the cellular stress response, apoptosis and pathways associated with wound healing in IEC. Consistently, IL-22 and epithelial STAT3 was found to be important in wound-healing experiments both in vivo and in cell culture experiments in vitro. In summary, our data suggest that intestinal epithelial STAT3 activation regulates immune homeostasis in the gut by promoting IL-22-dependent mucosal wound healing.

ER AND MITOCHONDRIAL STRESS SIGNALING UNDER CONDITIONS OF CHRONIC INTESTINAL INFLAMMATION

Messlik A [1], Rath E [1], Berger E [1], Fromme T [2], Klingenspor M [2], Hoogenraad J [3], Kim SC [4], Liu B [4], Sartor RB [4], and Haller D [1]*

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[2] Technische Universität München, Molecular Nutritional Medicine, ZIEL Research Center for Nutrition and Food Science, Freising-Weihenstephan, Germany

[3] La Trobe University, Department of Biochemistry, Melbourne, Victoria, Australia

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

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Background & Aim. The dramatic increase of chronically degenerative diseases in the industrialized world implies a complex interaction of host genetic predispositions and environmental factors. The gut acts as a highly selective barrier and communication organ between the luminal environment including food and bacterial components and the host responsible for the regulation of metabolic and immune functions. The peaceful and productive coexistence of the host with its intestinal microbiota is tightly controlled at various levels and an accumulating body of evidence suggests, that the failure of this homeostasis is thought to contribute to the development of inflammation-driven metabolic pathologies. Endoplasmic reticulum (ER) stress responses in intestinal epithelial cells (IEC) contribute to the initiation and perpetuation of chronic intestinal inflammation. We hypothesize that the failure of cellular stress communication between the ER communicate and the mitochondrion contribute to the development of chronic intestinal inflammation. The aim of this study was to characterize the interrelated roles of ER and mitochondrial stress responses in the intestinal epithelium of

patients with ulcerative colitis (UC) and two murine models of chronic, T cell-mediated colitis.

Methods and Results. Primary IEC were isolated from an adoptive transfer model of colitis, recombination activating gene deficient ($RAG2^{-/-}$) and $RAG2^{-/-}$ x IL-10 deficient ($IL\text{-}10^{-/-}$) mice reconstituted with $CD4^{+}$ T cells, as well as gnotobiotic $IL\text{-}10^{-/-}$ mice that were mono- or dual associated with colitogenic E. coli and Enterococcus faecalis. Proteome analysis of IEC using 2D-SDS PAGE and MALDI-TOF mass spectrometry identified 160 differentially regulated proteins clustered around the mitochondrial chaperonin 60 (cpn60) linking ER and mitochondrial unfolded protein response (UPR) under conditions of chronic inflammation. Western blot analysis of primary IEC from UC patients and murine models of colitis confirmed increased expression levels of the ER chaperone glucose regulated protein (grp)-78 and cpn60 associated with a complete loss of the mitochondrial creatine kinase (mtCK). To further study the signal integration of ER and mitochondrial stress responses, we selectively stimulated the IL-10 receptor reconstituted murine IEC line Mode-K with ER and mitochondrial UPR inducers tunicamycin (Tm) and mutant ornithine transcarbamylase (OTCΔ). Chromatin immunoprecipitation (ChIP) identified the ER-associated transcription factor ATF-6 to be recruited to ER (grp-78) and mitochondrial (cpn60, grp-75, ClpP) UPR gene promoters. In addition, Western blot and co-immunoprecipitation analysis revealed ER stress- induced UPR in purified mitochondria associated with the loss of mtCK. Interestingly, IL-10 inhibited ER and mitochondrial UPR at the level of transcription factor recruitment to gene promoters and protein expression including the loss of mtCK.

Conclusion. The proteomic and Western blot analysis of primary IEC from UC patients and murine models of chronic, immune- mediated colitis revealed strongly activated ER and mitochondrial UPR under conditions of chronic inflammation. Mechanistic studies demonstrated a tightly interrelated ER and mitochondrial stress response network integrated through ER-associated transcription factors including ATF-6. The anti-inflammatory immune mediator IL-10 inhibited ER and mitochondrial stress responses, suggesting cell stress regulation as a therapeutic target in the prevention of chronic intestinal inflammation.

PROGRAM
Friday,
April 24

THERAPEUTIC INTERVENTION IN PATTERN RECOGNITION OUTSIDE AND TLR – INFLAMMASOME CROSSTALK WITHIN IMMUNE CELLS

Carsten Kirschning

Institute of Medical Microbiology, University Clinic Essen, and Institute of Medical Microbiology, Immunology, and Hygiene, Technische Universität München, Germany, carsten.kirschning@uk-essen.de

Sepsis and septic shock upon Gram-negative or Gram-positive bacterial infection remain life-threatening syndromes despite antibiotics application and modern intensive care. Underlying hyperinflammation is triggered by cellular recognition of pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs). Therefore we evaluated selective TLR inhibition as clinical means. We found first that “immune suppressive” Rapamycin (inhibiting mTOR) which is applied for instance upon bone marrow transplantation and gynecologic tumors, while inhibiting TLR driven release of type I interferons, enhances cellular secretion of proinflammatory cytokines such as IL-12 and IL-1β. We also identified a TLR/STAT3/mTOR/PAI-2 signaling pathway that selectively inhibits specific inflammasomes. Our data assign an inflammation-promoting capacity to Rapamycin.

We then applied antagonistic monoclonal antibodies toward the extracellular domains of TLR2 or/and TLR4 to suppress the Jarisch-Herxheimer reaction that is elicited upon massive release of PAMPs from antibiotics treated bacteria. The selective blockade of TLR4 did not protect from Gram-negative sepsis, despite clearing antibiotics treatment. Dual TLR2/TLR4 blockade, however, was protective upon antibiotics therapy up to 4 hours after Gram-negative bacterial infection. According to these data might synchronous blockade of TLR2 and TLR4 result in effective therapeutic inhibition of Gram-negative sepsis pathology.

MUCOSAL IMMUNOLOGY

10^{00} – 11^{30} Chair: S. C. Bischoff, Department of Nutritional Medicine, University Hohenheim

J. Beisner, Dr. Margarete Fischer-Bosch-Insitute of Clinical Pharmacology, Stuttgart
α-Defensin HD-5 and WNT signalling transcription factor TCF-4 in pediatric patients with Crohn´s Disease

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

J.H. Niess, Department of Internal Medicine I, University of Ulm
The commensal gut flora drives the accumulation of CX3CR1 dendritic cells responsible for the induction of Th1 and Th17 responses in the large intestine

A. Mortha, Institute for Med. Microbiology & Hygiene, University of Freiburg
Development of natural killer cell-like lymphoid tissue inducer cells is driven by the commensal microflora

α-DEFENSIN HD-5 AND WNT SIGNALLING TRANSCRIPTION FACTOR TCF-4 IN PEDIATRIC PATIENTS WITH CROHN´S DISEASE

Julia Beisner, Gøri Perminow, Maureen Koslowski, Lars Gustav Lyckander, Eduard Stange, Morten H Vatn, Jan Wehkamp

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In adult patients small intestinal Crohn´s disease (CD) is characterized by a specific decrease of ileal Paneth cell α-defensins. In addition to NOD2, we previously identified a disturbance of the Wnt signalling transcription factor TCF-4 (Tcf7L2) as a major mechanism for Paneth cell α-defensin deficiency. Herein, we analyzed the human defensin 5 (HD5) and TCF-4 mRNA expression in a Norwegian cohort of pediatric patients with Crohn´s disease (treatment naïve) and age-matched controls. Untreated pediatric patients (<18 years, n=36) were included at the stage of diagnosis and age-matched controls were symptomatic non-IBD patients with histologically normal gut (n=29). In addition we assessed the influence of current inflammation using mucosal IL-8 as well as stool calprotectin levels. HD5 mRNA expression was significantly reduced in pediatric patients with ileal Crohn´s disease (L1+L3) compared to controls (p=0.022). We also found a significantly reduced expression of the transcription factor TCF-4 in pediatric Crohn´s disease patients with ileal involvement (p=0.0005). HD5 mRNA expression in the ileal mucosa was correlated with TCF-4 mRNA expression (r=0.499, p=0.0001). Importantly, the decrease of HD-5 and TCF-4 was neither correlated with mucosal IL-8 nor fecal calprotectin concentration and thus -independent of current inflammation -as observed in adults. In contrast to the small intestine, colonic Paneth cell HD5 mRNA expression was significantly up-regulated in case of colonic Crohn´s disease (L2). This (colonic) increase of HD5 correlated (r=0.481, p=0.020) with fecal calprotectin levels indicating that the presence of inflammation is triggering colonic Paneth cell metaplasia as an additional protection system.

In conclusion our data confirm a specific decrease of HD-5 and TCF-4 mRNA expression in pediatric patients with ileal Crohn´s disease (CD) which independent of current inflammation. In addition we show that inflammation itself can result in increased colonic HD5 due to metaplastic Paneth cells. These data further support the hypothesis of a paramount role of antimicrobial host defense in a pediatric population.

INDUCTION AND EXPANSION OF REGULATORY T CELLS AT MUCOSAL SURFACES

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Scientific Interests:
Mucosal immunity; autoimmunity, T cell tolerance, Tregs and effector cells, TH17, infection and inflammation

Regulatory T cells (T_{regs}) have potential anti-inflammatory effects and are likely to be important in the pathogenesis of chronic inflammatory bowel disease. However, the induction and expansion of T_{regs} at sites of mucosal inflammation is not yet fully understood and may involve antigen presentation by local dendritic cells and/or intestinal epithelial cells (IECs). To determine the unique ways of the gut to induce or expand T_{regs} we made use of a transgenic mouse model that is based on the specific expression of a model auto-antigen (influenza HA) in the intestinal epithelium (VILLIN-HA). Gut-associated dendritic cells (DCs) and intestinal epithelial cells (IECs) isolated from these mice were phenotypically and functionally characterized for the potential to interact with HA-specific T_{regs} *in vitro* and *in vivo*. Intestinal self-antigen expression leads to peripheral expansion of antigen-specific $CD4^+Foxp3^+$ T_{regs}. Although gut-associated DCs can induce antigen-specific $CD4^+Foxp3^+$ T cell proliferation, *in vivo* depletion of DCs did not preclude proliferation of these cells. Interestingly, antigen presentation by primary IECs is sufficient to efficiently expand antigen specific $CD4^+Foxp3^+$ T_{regs}. This is dependent on MHC class II but in contrast to DCs unlikely to require TGF-beta and retinoic acid. This study provides experimental evidence for a new concept in mucosal immunity: in contrast to current thinking, expansion of T_{regs} can be achieved independent of local DCs through antigen-specific IEC – T cell interactions.

COMMENSAL INTESTINAL BACTERIA AS POTENTIAL INDUCERS OR INHIBITORS OF CD4+ T-CELL INDUCED COLITIS

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Scientific Interests:

Dendritic cell tolerance; immune suppression; infection/ inflammation (TLR-ligands, viable bacteria); immune regulation in the intestine; autoimmunity, microbiota, probiotics.

The adoptive transfer of $CD4^+$ T-cells into immunodeficient hosts is a well known model of inflammatory bowel disease. The development of colitis in this model is associated with the accumulation of activated $CD4^+$ T-cells of the Th1 phenotype in the colonic lamina propria and results in severe colitis. Factors driving the Th1-biased T-cell responses like for example the $CD4^+$ T-cell subset and the bacterial antigen inducing inflammatory responses, as well as the APC stimulating the response are still not defined. We monocolonized germfree *Rag1*$^{-/-}$ with either *B. vulgatus* or *E. coli* mpk. In previous studies *B. vulgatus* revealed in the IBD model of *IL-2* deficient mice a protective, *E. coli* mpk a colitogenic potential. Additionally we analyzed specific pathogen free (SPF) and conventional (CV) *Rag1*$^{-/-}$ mice. The differently colonized *Rag1*$^{-/-}$ mice were transplanted with $CD4^+$ T-cells from healthy BL/6 donor mice and monitored for signs of intestinal inflammation. Furthermore histopathological changes and the phenotype of T-cells and DCs from cLP was analyzed. Monocolonized *Rag1*$^{-/-}$ mice did not develop colitis and only *E. coli* monocolonization led to T-cell repopulation. SPF Rag*1*$^{-/-}$ mice not show any signs of intestinal inflammation in contrast to CV *Rag1*$^{-/-}$ mice which developed severe colitis. Analysis of the intestinal microbiota revealed that induction of colitis in CV *Rag1*$^{-/-}$ mice was associated with an increase in *E. coli* but decrease in *Bacteroidetes* compared to SPF *Rag1*$^{-/-}$ mice and treatment of CV *Rag1*$^{-/-}$ mice with *B. vulgatus* was able to prevent induction of colitis. In line with this we were able to detect a significantly decreased *Bacteroides* to *E. coli* ration in children suffering from Crohn's disease. This data might indicate that the intestinal proportion of *E. coli* to *Bacteroidetes* might be an important factor in induction of prevention of colitis in a genetically predisposed host.

ROLE OF EPITHELIAL NF-κB ACTIVATION IN INTESTINAL IMMUNE HOMEOSTASIS

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Scientific Interests and Modelsystems:

Although the intestine contains billions 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, which prevents the interaction of microflora with mucosal immune cells. Disruption of the epithelial barrier and subsequent immune responses to the microflora are thought to be key factors in the development of Inflammatory Bowel Diseases (IBD). We are investigating the interplay between bacteria and 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. Genetic deficiency of the TLR adaptor protein MyD88 as well as germ-free conditions rescues $NEMO^{IEC\text{-}KO}$ mice from colonic inflammation, suggesting that TLR signaling initiated by intestinal microbiota is essential for colitis development in these mice. However, germ-free $NEMO^{IEC\text{-}KO}$ mice still exhibit increased apoptosis of IECs, indicating that loss of tolerance to intestinal microbiota is essential but nevertheless only secondary to epithelial barrier defects in the development of colon inflammation in $NEMO^{IEC\text{-}KO}$ mice. Although the intestinal epithelium is in close contact with microbiota, the NF-κB stimuli that prevent damage to the epithelial barrier have not been pinpointed. 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 and can be used to elucidate the cellular specificity of TLR signalling in intestinal immune homeostasis in mice.

THE COMMENSAL GUT FLORA DRIVES THE ACCUMULATION OF CX3CR1 DENDRITIC CELLS RESPONSIBLE FOR THE INDUCTION OF TH1 AND TH17 RESPONSES IN THE LARGE INTESTINE

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Scientific Interests:

Adaptive immunity, dendritic cells, mucosal immunity, commensals

Background & Aim: Dendritic cells (DCs) are a heterogeneous cell population that initiate immune responses and maintain tolerance by programming T cell reactivities. The ability of DCs in the colonic lamina propria (cLP) to induce the differentiation of regulatory or (pro)inflammatory T cell subsets was further determined.

Methods: DC subsets in the cLP of specific pathogen free (SPF) or germ-free (GF) CX3CR1/GFP mice were characterized.

Results: cLP DC can be divided into non-overlapping subsets that express CX3CR1 or CD103: 36% of the DC in the cLP express CD103, 35% of the cLP DC express CX3CR1 and 29% of cLP DCs lack CX3CR1 and CD103 surface expression. All cLP DC subsets express high MHC II and low levels of costimulatory (CD40, CD86, CD80) and coinhibitory molecules (PDL1/2). *Ex vivo* confocal microscopy demonstrated that $CX3CR1^+$ but not $CD103^+$ DC are reduced in the cLP of GF CX3CR1/GFP mice. In the terminal ileum $CX3CR1^+$ DC survey the intestine by extending processes into the lumen. In absence of the enteric flora the number of transepithelial processes is reduced. The colonization of mice with the enteric flora results in the local accumulation of $CX3CR1^+$ DC. In contrast, $CD103^+$ DCs appear in the cLP of mice with transfer colitis representing 62% of DCs in the inflamed lamina propria. Only $CD103^+$ but not $CX3CR1^+$ DCs induce the differentiation of FoxP3-expressing regulatory T cells. In contrast, $CX3CR1^+$ DCs drive the differentiation of TCR transgenice naïve T cells to TH1 and TH17 cells. In absence of CX3CR1 TH1 and TH17 cells are reduced in the lamina propria of homozygous CX3CR1/GFP mice.

Conclusion: Colonic DCs are a heterogeneous population that can be distinguished by CD103 and CX3CR1 expression. $CX3CR1^+$ DC induce the differentiation of TH1 and TH17 cells. Commensal bacteria-driven accumulation of $CX3CR1^+$ DC drives local TH1 and TH17 responses.

DEVELOPMENT OF NATURAL KILLER CELL-LIKE LYMPHOID TISSUE INDUCER CELLS IS DRIVEN BY THE COMMENSAL MICROFLORA

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It is widely believed that cues from the commensal microflora are needed to maintain intestinal epithelial homeostasis, but the molecular and cellular identities of these cues are unclear. We and others have recently identified a population of cells within the small intestine of mice that promote epithelial homeostasis by producing interleukin 22 (IL-22). This new lymphocyte population shows features of natural killer (NK) cells, as it expresses activating NK cell receptors such as *NKG2D*, *NK1.1* and *NKp46*. A major characteristic, linking these cells to lymphoid tissue inducer (LTi) cells, is their expression of and developmental dependence on the orphan transcription factor *Retinoic Acid Related Orphan Receptor* γ *t* (RORγt) and their independence of interleukin 15 (IL-15). Therefore, we chose to term those cells "NK-LTi cells". Our recent observations showed that the differentiation of NK-LTi cells is impaired in germ-free mice and could be re-gained by applying microbiota to those mice. Based on these data, we propose an instructive role for the commensal microflora in driving the differentiation of the NK-LTi cell population. To investigate this hypothesis, we analyzed LTi and NK-LTi cell populations in mice over time. Although NK marker-negative RORγt-expressing LTi cells were present even before birth (E18), the NK-LTi population emerged only 10 to 14 days later. Interestingly, in old mice (18 months) only few LTi cells were detectable whereas NK-LTi cells were dominant. IL-22 production within the LTi population was comparable at any timepoint analyzed, whereas I observed an increase in IL-22 expression in the newly appearing NK-LTi population within the first 4 weeks after birth. The expression of IL-22 in both LTi and NK-LTi cells of adult germ-free mice was strongly impaired. The increasing IL-22 production during postnatal development and in re-colonized germ-free mice, correlated with an increased epithelial expression of IL-22 response genes (i.e, RegIIIγ and RegIIIβ). Our data implies an instructive role of the commensal microflora for the development and effector functions of NK-LTi and LTi cells in the small intestine.

METABOLISM AND CHRONIC INTESTINAL INFLAMMATION: SIGNALS FROM THE FAT

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The fat tissue has primarily been described as an active metabolic organ. Data from the last decade not only indicate a strong interaction with the immune system, but furthermore suggest the fat tissue as an active member. In Crohn's disease, a chronic inflammatory disease of the intestine, a characteristic increase of the mesenteric fat tissue attached to the inflamed intestinal segments has been described. In addition, this fat tissue is creeping around these inflamed areas. Why this hypertrophy of the mesenteric fat occurs builds the focus of current research. Based on experimental data by our group and others, a working hypothesis can be suggested: Genetic predisposition leads to altered adipokine and cytokine production in the mesenteric fat and cells of the adjacent intestinal mucosa. This results in an infiltration of immune cells into the lamina propria with subsequent increased local cytokine production further fueling adipokine release in the nearby adipose tissue. In consequence, during contact to yet not completely identified environmental factors the resulting immune response is disproportional. Mucosal cytokine production and the enhanced adipokine bacterial translocation due to disruption of the mucosal integrity additionally induce adipokine release by the mesenteric tissue and might lead to the hypertrophy of the mesenteric fat in CD.

PROBIOTICS

14^{30} – 16^{15} Chair: W. Kruis, Internal Medicine, Evang.Krankenhaus Kalk

K. Fink, Institute of Med. Microbiology & Hygiene, University Tübingen
Increased mortality of E.coli Nissle 1917 mono-colonized RAG1 deficient mice upon $CD4^+$ T cell transfer

J. Preising, Institute for Microbiology and Biotechnology, University of Ulm
Anti-inflammatory activity of a B. bifidum strain in RAG1 $^{-/-}$ colitic mice

A. Sturm, Division of Hepatology & Gastroenterology, Charité Berlin
Escherichia coli Nissle 1917 induces apoptosis of peripheral $\gamma\delta$ T cells

C. Rasche, Department of Dermatology and Allergy, Charité Berlin
Modulation of the allergic response by inactivated non-pathogenic E. coli Nissle

T.A. Ölschläger, Institute for Molecular Infection Biology, University of Würzburg
The flagella of the probiotic Escherichia coli strain Nissle 1917 – a multipurpose tool

C. Pöhlmann, Institute of Med. Microbiology & Hygiene, TU Dresden
Secretion of biologically active recombinant IL-10 via a modified hemolysin transport system in Escherichia coli Nissle 1917

S. Hummel, Department of Infectiology, Centre for Molecular Biology of Inflammation (ZMBE), Münster
Gram-negative and gram-positive probiotic bacteria do it differently

INCREASED MORTALITY OF *E. COLI* NISSLE 1917 MONO-COLONIZED RAG1 DEFIZIENT MICE UPON $CD4^+$ T CELL TRANSFER

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Scientific Interests and Modelsystems:

interaction of host immune system and intestinal flora; adoptive T cell transfer in Rag1-/- mice; DSS colitis; TLR knockout mice

E. coli Nissle 1917 (EcN) is a well defined probiotic *E. coli* strain, which is effective in maintaining remission in ulcerative colitis {Rembacken, 1999 160 /id}. The aim of this study was to explore safety aspects of this frequently used probiotic strain in immune deficient hosts. Therefore, germfree *Rag1*$^{-/-}$ mice were mono-colonized with EcN. Upon transplantation of naïve $CD4^+$ T cells of healthy C57BL/6 donor mice the *E. coli* Nissle mono-colonized *Rag1*$^{-/-}$ mice died. Analysis of bacterial load in the peripheral organs revealed a massive translocation of EcN and increased TNF-□ serum levels. In contrast, SPF *Rag1*$^{-/-}$ mice were protected even though the numbers of bacteria in the intestine did not differ. Furthermore, EcN mono-colonized C57BL/6 mice showed no increased mortality rate indicating that $CD4^+$ T cells were essential either for the inhibition of translocation or for the clearance of EcN from the peripheral organs. The translocation and dissemination are EcN specific effects, as they were not observed in germfree *Rag1*$^{-/-}$ mice mono-colonized with *E. coli* mpk, a commensal *E. coli* strain isolated from the murine intestine. Additionally, the dissemination of EcN in mono-colonized *Rag1*$^{-/-}$ mice was not flagella dependent, as the translocation of EcN □*fliC* and EcN □*flgE* to peripheral organs was comparable to the translocation of wildtype EcN. Surprisingly, transplantation of $CD4^+$ T cells in germfree *Rag1*$^{-/-}$ mice before they were mono-colonized with EcN did not increase the mortality rate and only few EcN disseminated into the peripheral organs. This also indicates that $CD4^+$ T cells seem to be essential to inhibit translocation of EcN or to clear disseminated EcN.

ANTI-INFLAMMATORY ACTIVITY OF A *B. BIFIDUM* STRAIN IN RAG1$^{-/-}$ COLITIC MICE

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Bifidobacteria have several beneficial effects for their host. Strain-dependent anti-inflammatory activity of various strains and species of bifidobacteria was previously shown by our group in *in vitro* experiments using LPS-challenged HT-29 cells. Here we analyse the anti-inflammatory effects of a strain of *B. bifidum* with good adhesive properties to intestinal epithelial cells in the Rag1$^{-/-}$ mouse model of murine colitis. The transfer of CD4^{+} T-cell populations from wild type C57BL/6J into congenic Rag$^{-/-}$ mice leads to the development of colitis mediated by T-helper type 1 cells due to the lack of mature regulatory T-cells in these mice.
In a placebo-controlled set up one group of Rag1$^{-/-}$ mice (Rag1^{tm1Mom}; $n = 4$) received one oral dose of our probiotic *B. bifidum* strain (2×10^{9} cfu per animal) in PBS followed immediately by transfer of CD4^{+} T-cells. Two control groups received placebo of which one group also was transferred with CD4+ T-cells to induce colitis. Feeding with the probiotic and placebo was continued three times a week and weight was recorded for 34 days when all animals were sacrificed. The anti-inflammatory effect of feeding the *B. bifidum* strain was assessed by measuring weight and length of dissected colons, histology scores of colonic tissue samples, and qRT-PCR for various pro-inflammatory cytokines. Additionally, the composition of the bacterial flora of all mice was analysed by fluorescence *in situ* hybridisation combined with flow cytometry.

ESCHERICHIA COLI NISSLE 1917 INDUCES APOPTOSIS OF PERIPHERAL γδ T CELLS

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Background: Since γδ T cells specifically recognize microbial antigens such as pathogen-associated molecular patterns (PAMPs) without the need of antigen presenting cells or MHC molecules, they play a central role in the regulation of inflammatory processes. We previously demonstrated that the probiotic E. coli Nissle strain Nissle (EcN) ameliorates intestinal inflammation by inhibiting peripheral T cell expansion, prompting us to investigate the effect of EcN on γδ T cells. *Methods:* Peripheral blood mononuclear cells from healthy volunteers were stimulated with Isopentylpyrophosphat and IL-2 and cultured in the presence or absence of 25% (vol/vol) steril filtered EcN supernatants (EcN-SN) for 72h. T cell populations were identified by flow cytometric analysis or for some experiments γδ T cells were negatively sorted using magnetically labeled anti ab TCR antibodies. Flow cytometry was used to assess apoptosis (Annexin V) and necrosis (PI) of γδ T cells (anti γδ TCR) as well as their Fas, Fas-L and TNF-a receptor expression profiles. To determine apoptotic pathways, stimulated cells were cultured with caspase inhibitors and blocking anti Fas antibody followed by flow cytometry analysis. The mitochondrial membrane potential was determined by rhodamin123 staining. *Results:* Compared to controls, EcN significantly increased γδ T cell apoptosis by 36.9±5% ($p<0.05$) and necrosis by 25.3±3% ($p<0.05$). In contrast, EcN did not induce ab T cell death. EcN increased Fas-L expression of γδ T cells by 34.3±6%, while TNF-a receptor expression dropped by 42.1±4% and Fas expression remained unchanged. EcN-induced γδ T cell death was significantly inhibited by the ubiquitous caspase inhibitor zVAD, the caspase inhibitors-3, -8 and -9, and blocking Fas-L antibodies. Examining the intrinsic apoptotic pathway, EcN decreased the mitochondrial membrane potential of γδ T cells.
CONCLUSION: Our data demonstrate that EcN potently induces γδ T cell death via caspase and FasL-dependent pathways. Inflammatory bowel diseases are characterized by an uncontrolled immune response against the indigenous gut flora. γδ T cells are strongly involved in the recognition of microbial antigens and the perpetuation of inflammatory processes. Thus, the blockage of this pathway by EcN might further explain its beneficial effects in inflammatory bowel diseases and help to better understand the role of γδ T cells in mucosal inflammatory processes.

MODULATION OF THE ALLERGIC RESPONSE BY INACTIVATED NON-PATHOGENIC *E. COLI* NISSLE

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Background Allergic disorders are strongly influenced by environmental factors such as microbial antigens. Especially probiotic bacteria have been proposed to have modulating effects on the development and outcome of the allergic disease. The purpose of this study was to study the role of inactivated non-pathogenic E. coli strain Nissle (EcN) on the phenotype and function of T- and B-cells. *Method* T- and B-lymphocytes from grass-pollen allergic (A, n=10) and non-allergic patients (NA, n=19) were co-stimulated with inactivated EcN and grass-pollen allergen. We analysed the expression of CD23 and co-stimulatory molecules but also the intracellular production of IL-4 and IFNγ by direct ex-vivo flow cytometry. *Results* Allergic patients showed a significant up-regulation of CD23 expression after stimulation with allergen ($p<0.05$). By contrast, CD23 expression was significantly reduced after stimulation with EcN plus allergen ($p<0.05$). Also, CD86 but not CD80 expression was significantly increased after stimulation with allergen and with EcN in both groups ($p<0.05$). The cytokine-patterns of CD69-positive T-lymphocytes of the allergic individuals showed a TH2-dominated response after allergen stimulation (IFNγ/IL-4 ratio 0.3). By contast, stimulation with EcN resulted in a TH1-dominated response (IFNγ/IL-4 ratio EC $3.0_{(A);}$ $1.75_{(NA)}$). Interestingly, the allergen-induced TH2-dominated response was shifted into a TH1-dominated response by the addition of EcN (IFNγ/IL-4 ratio $3.67_{(A);}$ $2.83_{(NA)}$) due to an increase of IFNγ-production ($p<0.05$). *Conclusion* Our data show that non-pathogenic EcN modulate the allergic immune response by the alteration of CD23 and co-stimulatory molecule expression. Additionally, EcN modulate the allergen specific immune response by promoting a TH1-dominated immune response in conjunction with allergen challenge, as well. The data suggest that non-pathogenic EcN may play a role in the prophylaxis and treatment of allergic disorders.

THE FLAGELLA OF THE PROBIOTIC *ESCHERICHIA COLI* STRAIN NISSLE 1917 - A MULTIPURPOSE TOOL

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Scientific Interests and Modelsystems:

1) Adhesion and invasion of intestinal and extraintestinal pathogenic enterobacteria. Modelsystems are human gut and bladder epithelial cell lines as well as human gut biopsies

2) Elucidation of the molecular mechanisms of the *E. coli* strain Nissle 1917 involved in its probiotic actions. Modelsystems are human epithelial cell lines, human gut biopsies and the mouse.

The probiotic *Escherichia coli* strain Nissle 1917 (EcN) of serotype O6:K5:H1 is effective in treatment of various intestinal diseases e.g diarrhea and keeping colitis ulcerosa (CU) patients in remission. Its flagella is not only essential for motility but rather a multipurpose tool. We could demonstrate (1) the flagella to be also responsible for efficient adherence to cryosections of crypts from human gut biopsies in contrast to type 1 pili and F1C fimbriae.

(2) Furthermore, induction of human beta defensin 2 (hBD2) expression in Caco-2 cells is depending on FliC, the major flagellar protein. Evenso the uropathogenic *E. coli* strain CFT073 harbours an identical *fliC* gene and therefore an identical FliC protein as EcN this strain is a much weaker hBD2 inducer. SDS PAGE revealed a slightly higher electrophoretic mobility for the FliC-CFT073 protein. The difference in electrophoretic mobility was not observed after FliC expression in an in vitro transcription-translation system. These results might indicate posttranslational modification of FliC-EcN in vivo. MALDI-ToF analysis of both

FliCs showed identity for all detected trypsin fragments. However, one trypsin fragment could not yet be identified.

(3) Because EcN is not effective in treatment of Crohn's disease (CD) in contrast to its effectiveness in CU and because many CD patients show defects in defensin induction, we started to construct recombinant EcN strains expressing a human defensin. Such recombinant EcN strains could be applied as delivery vehicles for defensins in CD patients. In a first approach we cloned the human alpha-defensin 5 (hAD5), because this is the only defensin produced in an inactive proform. In fact, the recombinant EcN strain was shown to express the inactive proform of hAD5. Release of the recombinant hAD5 from EcN in vivo might be achieved by secretion via the flagellar secretion system or by lysis of the recombinant EcN cells caused by the gut environment and subsequently also by the released and proteolytically activated hAD5. For export via the flagellar secretion system we constructed a *fliC*, *fliD* EcN double mutant and ligated the hAD5 gene into the two different secretion vectors (pSRP18/0; pKS073) kindly provided by B. Westerlund-Wikström.

These ongoing studies provide evidence for the importance of the flagella in EcN's probiotic activity.

SECRETION OF BIOLOGICALLY ACTIVE RECOMBINANT IL-10 VIA A MODIFIED HEMOLYSIN TRANSPORT SYSTEM IN *ESCHERICHIA COLI* NISSLE 1917

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Gram negative bacteria use different transport systems for secretion of proteins into the extracellular environment. The aim of our project is to use the probiotic *E. coli* strain Nissle 1917 (EcN) as a vehicle for intestinal synthesis of therapeutic molecules. We focused on the anti-inflammatory cytokine IL-10 which has emerged as a promising option for the treatment of inflammatory bowel diseases (IBD). Several clinical trials have recently validated EcN as a therapeutic alternative to standard medication for the treatment of IBD. EcN is considered a safe organism even for infants. Due to its beneficial traits and good colonization properties, EcN is an ideal candidate for the development of a microbial drug for delivery of recombinant therapeutic molecules in the intestine. The *E. coli* a-hemolysin transport system was modified by inserting a linker region between murine IL-10 (mIL-10) and the hemolysin A signal peptide. The linker region contains potential cleavage sites for the outer membrane protease T (OmpT) of *E. coli*. Thus, processing of the signal peptide fused to the mIL-10 C-terminus is achieved during translocation of mIL-10 across the outer membrane. Successful cleavage of the hemolysin A signal peptide was confirmed by Western Blot analysis of sterile filtered culture supernatant of the linker plasmid transformed EcN strains. By using conventional ELISA techniques, EcN cells secreted up to 60 ng recombinant mIL-10 per milliliter culture supernatant. The biological activity of the secreted mIL-10 was verified by using an IL-10 dependent murine mast cell line (D36 cell assay). Statistical analysis was performed with a student t test (*: $p<0,01$).

GRAM-NEGATIVE AND GRAM-POSITIVE PROBIOTIC BACTERIA DO IT DIFFERENTLY

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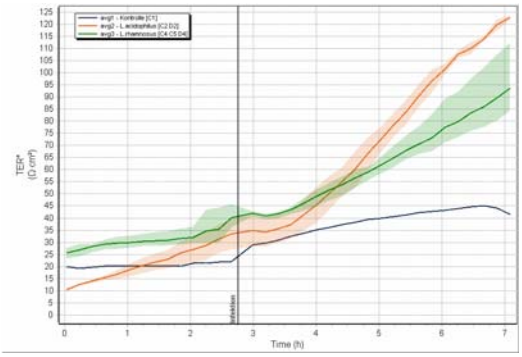

Impact of *Lactobacilli* on TER of T84 monolayer (cellZscope, Nano-analytik, online measurement)

New strategies for the treatment of gastrointestinal diseases, junctional complex, T84 cells

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 and contributing to inflammatory bowel diseases (IBD). Taking into account that IBD may develop after defects of barrier function and based on Affymetrix microarray data we focussed on genes encoding for tight junction (ZO-2) and adherence junction (β-catenin and E-cadherin) proteins modulated by different protein kinase C (PKC) isoforms. We used the transepithelial electrical resistance (TER) of a T84 monolayer after co-incubation with probiotic bacteria as a read out system for the paracellular permeability. Generally our data indicate that the barrier function is subject to different regulation of adherence junction proteins by Gram-postive and tight junction proteins by Gram-negative probiotic bacteria. This striking difference is also true for the activity (i.e. phosphorylation) of different PKC isoforms (Gram-positive: [novel PKCδ] or Gram-negative: [atypical PKCζ]). This study revealed cellular responses of IECs specifically induced by the probiotic *E. coli* Nissle or various *Lactobacilli*. Further insight into the molecular mechanisms will foster the development of new strategies for the treatment of gastrointestinal diseases i.e. IBD.

PROBIOTICS IN THE PREVENTION OF ATOPIC DISEASES

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Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. First well-conducted clinical studies with probiotics date back to the late eighties and has been conducted in children suffering from acute viral gastroenteritis. Important immunoregulatory role of gut microbiota has recently been shown. Tolerance to dietary antigens, oral tolerance, is not achieved in germ-free environment. Both development and function of T regulatory cells are dependent on gut microbiota. These regulatory T cells are mandatory for keeping responses of T helper (Th) cells in equilibrium. Escalation of Th1 responses would otherwise result in e.g. Crohn's disease and that of Th2 responses would cause allergic inflammation. Probiotics have many potential mechanisms of action in the gastrointestinal tract to suppress both of these Th responses, Preventive effects of certain probiotic strains on allergic diseases have been studied during the last decade in several studies. The results have so far been promising but partly conflicting in the prevention of atopic eczema. Future studies are needed to show whether prevention of atopic diseases could be an indication for probiotics.

PROGRAM
Saturday, April 25

ANALYSIS OF THE HUMAN MICROBIOME

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The human intestinal tract harbours a complex microbial ecosystem which plays a key role in nutrition and health. During the last century the development of anaerobic culturing techniques allowed the characterization of the human faecal microbiota. Many of the microorganisms in the human gut are fastidious and will only be cultivable when their specific requirements are known. Consequently increases in the number of microbiota identified were low when culturing was the only method available. The development of culture-independent tools such as PCR and sequencing of the 16S ribosomal RNA gene during the past decades has resulted in a dramatic increase in the number of gut microbes identified, allowing a complete reassessment of the microbial diversity of the human gut. Based on molecular analysis and confirmed by other approaches such as fluorescent in situ hybridisation (FISH), 80% of the phylotypes observed in faecal microbiota of healthy young adults belong to four phylogenetic groups: the Gram negative *Bacteroides* cluster, the low-GC Gram positives of the phylum Firmicutes, including the *Clostridium coccoides* phylogenetic group (cluster XIV) and the *Clostridium leptum-Faecalibacterium prausnitzii* (cluster IV), and the high-GC Gram positives of the *Bifidobacterium* and the *Collinsella-Atopobium* groups.

While molecular tools such as PCR, cloning and sequencing have their own biases and limitations, they support the recognition of a substantially wider diversity of species or phylotypes, the majority of which bear no cultured representative in the databases. Culturable representatives are only available for a minority (20%) of the phylotypes identified by culturing independent methods. This is especially true for the Firmicutes. The proportion of yet unrecognized and unculturable gut microbial species increases from birth to old age. Comparative analysis of several 16S ribosomal DNA based inventories and high throughput methods inform us that the dominant faecal microbiota is essentially specific to its host at the species level, it is resistant to modification and shows a marked resilience following stress. Although the human faecal microbiota is diverse in its microbial composition its functionality is expected to be homogeneous between individuals. However it is not yet clear at which level, genome, proteome or metabolome, this functional homogeneity or functional core can be identified. The current status of

investigations into the human faecal metagenome will be reviewed, which will cover healthy as well as inflammatory bowel disease patients. For example a metagenomic approach has revealed a reduced complexity of the bacterial phylum Firmicutes as a signature of the faecal microbiota in CD and this loss of diversity was confirmed by FISH on an individual level (2). It is further noteworthy that bacteriophages which outnumber the bacteria by a factor of 10, are hypothesized to exert a strong influence on bacterial diversity and population structure, and are probably involved in dysbiosis by destabilising bacterial communities.

A long coevolutionary process has led to mutualistic interactions between the gut microbiota and the host. The continuous regeneration and proliferative activity of the intestinal epithelium are modulated by the microbiota or its metabolites. A better understanding of the microbiota's contribution to human health requires characterization of microbial molecular signals driving interactions with the host. Beyond sequence-based human intestinal microbiome explorations, metagenomic libraries facilitate functional investigations. Functional screening of the metagenomic libraries allows for the identification on unexplored genomic resources some of which are likely to be highly relevant to our understanding of the host microbe interaction. Our current analyses in combination with transposon mutagenesis allowed us to identify various genes encoding specific enzymes (e.g. ß-glucuronidases), encoding biosynthetic pathways (e.g. riboflavinbiosynthesis) or epithelial cell growth modulation (1) .

In the future these investigations will be complemented by more in-depth knowledge of the genomic resources of a reference set of micro-organisms. This reference set of micro-organisms is analysed as part of a concerted effort to sequence the human metagenome, the ensemble of the genomes of human-associated micro-organisms. One of these projects, MetaHIT (Metagenomics of the Human Intestinal Tract for Health), aims to give an unprecedented view of the gut microbiota and amongst others their variability, and identification of microbial signatures of prognostic and diagnostic value. These approaches promise to identify the most redundant genomic traits of the human intestinal microbiota, thereby identifying the functional balance of this organ. Ultimately it will support the concept of a functional core within the intestinal microbiome.

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2. **Manichanh, C., L. Rigottier-Gois, E. Bonnaud, K. Gloux, E. Pelletier, L. Frangeul, R. Nalin, C. Jarrin, P. Chardon, P. Marteau, J. Roca, and J. Dore**. 2006. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut **55**:205-211.

MICROBES AND HOST

10^{00} – 11^{30} Chair: J.-S. Frick, Institute of Med. Microbiology & Hygiene, University Tübingen

J. Pott, Inst. for Medical Microbiology & Hospital Epidemiology, Hannover Medical School
Invasion-dependent recognition of Mycobacterium avium subsp. paratuberculosis

J. Vogel-Scheel, German Institute of Human Nutrition, Potsdam- Rehbrücke
Analysis of physiological status of Escherichia coli in different regions of the gastrointestinal tract in a gnotobiotic mouse model

R. Plickert, Institute of. Microbiology & Hygiene, Charité Berlin
MyD88 mediated TLR9 sensing modulated by TLR9 antagonist in GvHD

D. Mailänder-Sánchez, UK Tübingen
Impact of lactobacillus species on localised Candida albicans infection and the mucosal innate immune response

C.J. Chou, Nestlé Research Center, Lausanne, Switzerland
Gut microbiota modulation improves glucose tolerance of mice with insulin resistance

T. Werner, Biofunctionality, Centre for Diet and Disease, TU München
Reduction of dietary iron and systemic iron replenishment inhibit the development of chronic ileitis in TNF $^{\Delta ARE/WT}$ mice targeting endoplasmic reticulum stress mechanisms in intestinal epithelial cells (IEC)

INVASION-DEPENDENT RECOGNITION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* BY INTESTINAL EPITHELIAL CELLS

Johanna Pott, Mathias W. Hornef

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Scientific Interests and Modelsystems:
Mycobacterium avium subsp. *paratuberculosis* ; John´s disease; Crohn´s disease; innate immune recognition by intestinal epithelial cells

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of John's disease, a highly prevalent chronic intestinal infection in ruminants. The microbial pathogenesis of MAP infection has recently attracted significant attention due to a possible association with the human enteric inflammatory disease *Morbus Crohn*. Infection is acquired by the fecal-oral route prompting us to study the interaction of MAP with differentiated intestinal epithelial cells. In contrast to other opportunistic mycobacteria or *M. bovis,* MAP induced significant epithelial activation in an Erk-dependent but NF-κB independent manner. MAP was rapidly internalized and accumulated in a late endosomal compartment. Surprisingly, MAP recognition was completely internalization-dependent and inhibition of Rac-dependent bacterial uptake abolished epithelial activation. In accordance, the analysis of the innate immune recognition of MAP by epithelial cells revealed significant involvement of the intracellularly localized receptors TLR9 and NOD1 and signalling through the adaptor molecules MyD88 and RIP2. The invasion-dependent innate immune activation of MAP might contribute to the intestinal pathology.

ANALYSIS OF PHYSIOLOGICAL STATUS OF *ESCHERICHIA COLI* IN DIFFERENT REGIONS OF THE GASTROINTESTINAL TRACT IN A GNOTOBIOTIC MOUSE MODEL

Jacqueline Vogel-Scheel, Carl A. Alpert, Wolfram Engst, Gunnar Loh, Michael Blaut

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The in vivo growth conditions of intestinal bacteria differ from those in cultivation media. We are interested in elucidating the mechanisms enabling bacteria to adapt to the environment in the intestine. For our investigation, we used mice monoassociated with *Escherichia coli* MG1655 as a simplified model.

We hypothesized that bacterial adaptation is reflected by a modified bacterial protein expression. Two-dimensional difference gel electrophoresis followed by electro-spray ionization-tandem mass spectrometry was used to identify proteins differentially expressed in MG1655 recovered from the cecum of monoassociated mice and in MG1655 grown under anoxic conditions on a complex medium reflecting the substrate composition of the animal diet. We detected 69 proteins differentially expressed under *in vivo* and *in vitro* conditions. Most of the identified proteins belong to the central energy and protein metabolism and we conclude from the expression pattern that *E. coli* uses an extended spectrum of substrates under *in vivo* conditions. The down-regulation of proteins involved in peptide uptake suggests that peptide availability in the cecum is lower than in cultivation media. We hypothesize that the same applies to the availability of nucleotides or their precursors because under in vivo conditions, enzymes of the purine and purimidine synthesis were up-regulated 7.4 fold and 13.3 fold, respectively. We observed a 3.5 fold upregulation of the putative methylglyoxal reductase YDJG which has been suggested to be involved in puryvate formation from dihydroxyacetone phosphate via methylglyoxal under fasting conditions. A 3 fold upregulation of N-acetylneuraminate lyase in the cecum involved in the degradation of mucin constituents indicates that MG1655 utilizes host-derived substrates. Our results indicate intestinal substrate availability is a key factor that influences the expression of abundant proteins, while host factors appear to be less important.

MyD88 Mediated TLR9 Sensing Modulated by TLR9 Antagonist in GvHD

Rita Plickert, [1] Markus M. Heimesaat, [1] Axel Nogai, [2] Stefan Bereswill, [1] André Fischer, [1] Christoph Loddenkemper, [3] Ulrich Steinhoff, [4] Eckhard Thiel, [2] Marina Freudenberg, [5] Ulf B. Göbel [1], Lutz Uharek [2]

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Graft-versus-host-disease (GvHD) is a major cause for morbidity and mortality after allogeneic stem cell transplantation. Acute intestinal GvHD is mediated by bacterial sensing via toll-like-receptors (TLR), but the underlying immune pathology is yet poorly understood. In a murine stem cell transplantation model based on reduced intensity conditioning, we studied the role of TLR9 on intestinal GvHD. Following transplantation, wild type mice displayed severe intestinal tissue damage, accompanied by elevated numbers of T-cells, neutrophils, apoptotic and proliferating cells in the colon (as shown by in situ immunhistochemistry staining). In TLR9$^{-/-}$ as well as MyD88$^{-/-}$ mice, however, the numbers of T-cells, neutrophils and apoptotic cells in the colon were significantly lower as compared to wildtype mice after transplantation, resulting in amelioration of the tissue damage and improved survival.

Administration of a synthetic TLR9 antagonist (iODN2088 i.p.) to wild type mice after transplantation led to similar results as shown in TLR9$^{-/-}$ mice pointing towards a pivotal role of TLR9 sensing in intestinal GvHD. A synthetic TLR9 antagonist may offer a novel option for treating and preventing GvHD in the future.

IMPACT OF LACTOBACILLUS SPECIES ON LOCALISED *CANDIDA ALBICANS* INFECTION AND THE MUCOSAL INNATE IMMUNE RESPONSE

Mailänder-Sánchez, Daniela; Wagener, Jeanette and Prof. Dr. Schaller, Martin

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Scientific Interests:
host pathogen interaction

Modelsystems:
in vitro models of reconstituted human oral epithelium (RHE)

The commensal yeast *Candida albicans* is the main-agent of fungi-caused diseases in humans. Several probiotic *Lactobacillus* species are known, that exert inhibiting and/or protective effects on *C. albicans* and other infections *in vivo* and *in vitro*. Therefore we choose *L. rhamnosus* GG (LGG) to investigate the role of this species on localised *C. albicans* infections, using a model system of localised candidiasis to analyze a number of different aspects of host/*Candida* interactions.
Preliminary results indicate a protective role for LGG in our model. RHEs treated with *Candida* and LGG showed significantly lower levels of lactate dehydrogenase (LDH), used as a marker of cell damage, compared to LDH-levels of RHEs treated only with *Candida*. This can also be confirmed by light microscopy where epithelium with co- cultured *Candida* and *Lactobacilli* resemble untreated controls whereas epithelium cultured with *Candida* alone is strongly damaged. Furthermore, LGG seems to reduce the proinflammatory cytokine response of the RHEs towards *Candida* infection. These effects can also be obtained by the use of heat inactivated *L. rhamnosus GG*. Additionally the expression of TLR-2 and TLR-4 seems to be altered by the use of LGG.
L. rhamnosus GG exert protective effects in a model of localized oral candidiasis that are possibly mediated via TLR.

GUT MICROBIOTA MODULATION IMPROVES GLUCOSE TOLERANCE OF MICE WITH INSULIN RESISTANCE

Rabot Sylvie[2], Gérard Philippe[2], Bruneau Aurélia[2], Blancher Florence[1], Membrez Mathieu[1], Bibiloni Rodrigo[1], Rezzonico Enea[1], Berger Bernard[1], Krause Lutz[1], Moser Mireille[1], Macé Katherine[1], Chou Chieh J.[1]

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Scientific Interests:

Impacts of the gut microbiota on body weight and insulin resistance

Modelsystems:

in vitro and animal models

In our previous study, administration of broad range antibiotics norfloxacin and ampicillin drastically reduced the number of intestinal bacteria and ameliorated multiple metabolic abnormalities such as fasting hyperglycemia, oral glucose intolerance and low hepatic glycogen storage in ob/ob mice. The beneficial effects of the treatment were independent of food intake or body weights. To further examine the impacts of gut microbiota reduction on the development of diabetes, germ-free C57BL/6J mice residing in an isolator were challenged with a sterile high fat diet for 11 weeks. In contrast to the conventional counterparts, germ-free mice were resistant to diet-induced obesity and insulin resistance. Plasma TNFα level was significantly reduced in the germ-free mice suggesting that the presence of gut microbiota is a contributing factor to low grade inflammation in the DIO mice.

To demonstrate causal effect of gut microbiota on the development of insulin resistance, we inoculated germ free C57BL/6J mice with the cecal contents from DIO donors. From a cohort of 40 DIO mice, an obese mouse with severe hyperglycemia (responder) and a lean mouse with mild hyperglycemia (non-responder) were selected as donors of cecal contents. Ex-germ free mice who received the cecal content from the responder were more glucose intolerant than those who received the cecal content from the non-responder. In a parallel study, DIO mice received non-absorbed antibiotics polymyxin B and neomycin (poly/neo) showed significantly altered cecal microbiota profile. Two wks poly/neo treatment did not improve the glucose tolerance of DIO mice. Surprisingly, the treated DIO mice became more glucose tolerant by the end of 4 wks washout period. The altered intestinal microbiota profile remained stable in the washout period and correlated with improved glucose tolerance in DIO mice. In summary, our data indicate that insulin sensitivity is a transmissible trait through the gut microbiota inoculation. Compositions of microbiota that dwells in the GI tract affect inflammatory responses and influence the development of insulin resistance in mice.

REDUCTION OF DIETARY IRON AND SYSTEMIC IRON REPLENISHMENT INHIBIT THE DEVELOPMENT OF CHRONIC ILEITIS IN TNF$^{\Delta ARE/WT}$ MICE TARGETING ENDOPLASMIC RETICULUM STRESS MECHANISMS IN INTESTINAL EPITHELIAL CELLS (IEC)

Werner T [1], Wagner S J [1], Clavel T [1], Schuemann K [2], Haller D [1]

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Endoplasmic reticulum (ER) stress in IEC contributes to the development of chronic intestinal inflammation. Our aim was to characterize the role of dietary and systemic iron on the regulation of ER-stress in experimental ileitis.

Histological scoring (0-12) revealed that iron-low fed TNF$^{\Delta ARE/WT}$ mice (score 2.30+/-0.76) were almost completely protected from the development of severe ileal inflammation in contrast to iron-adequate fed TNF$^{\Delta ARE/WT}$ mice (score 8.30+/-0.91). This suggests a pathological role for luminal enteric iron in chronic ileitis. Systemic iron replenishment of iron-low fed TNF$^{\Delta ARE/WT}$ mice by intraperitoneal injections (90µmol iron/week) did not reverse the protective effect of iron-low feeding (score 1.67+/-0.20). Western blot and immunohistochemical analysis from inflamed (iron-adequate) versus non-inflamed (iron-low) primary IEC and ileal tissue sections revealed down-regulation of ER-associated stress mechanisms, including the expression of the glucose regulated protein (grp)-78. Chromatin-immunoprecipitation analysis identified XBP-1 and NRF-2 recruitment to the grp-78 promoter in the murine small IEC line Mode-K after stimulation with TNF and iron, confirming a mechanistic link between iron and ER-stress signaling.

This study clearly demonstrates the protective effect of low dietary iron and systemic iron replenishment on chronic experimental ileitis in TNF$^{\Delta ARE/WT}$ mice targeting iron as an essential modulator of ER-stress in the intestinal epithelium.

POSTER

1 REDUCTION OF GUT-CAUSED HALITOSIS WITH PROBIOTIC *E.COLI* NISSLE 1917 - FIRST FINDINGS

S. Straub[1], K. Andraczek[1], , C. Wolff[2], K. Richter[3], A. Landrock[3], L. Schulze[2], J. Henker[1]

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INTRODUCTION: In about 10% of cases, bad breath is not of oral origin. Volatile compounds produced by bacterial processes in the intestines and transported by blood to the lungs or the oral glands may also be responsible for halitosis.

AIMS & METHODS: This pilot study aimed at investigating the effect of the probiotic *Escherichia coli* Nissle 1917 (EcN) on gut-caused halitosis in healthy subjects with no significant dental report. Seven persons with oral malodor received two capsules EcN (Mutaflor®; Ardeypharm GmbH, Herdecke, Germany) per day, each containing 2.5-25 x 10Exp9 colony forming units over a period of 28 days. Oral breath was consequently assessed organoleptically using a 0-to-5 scale (from 0 = no odor detectable to 5 = extremely strong odor). In addition, exhaled air and tongue smear of the volunteers were analyzed by high sensitive GC/MS analysis.

RESULTS: Scores of organoleptic assessment reaching 3 to 4 before treatment were consistently reduced to 0 to 2. By GC/MS analysis Indole, a compound with proven importance in oral malodor, and phenol were found consistently at remarkably high levels. Following the four-week intake of the probiotic these compounds were considerably reduced in tongue smear specimen. Indole showed a reduction ranging between 81.9% and 99.9%. Phenol values were also noticeably reduced (median 79.6%, minimum 24.5%, maximum 99.7%)

CONCLUSION: EcN seems to reduce the symptoms of halitosis in seven dentally healthy individuals according to organoleptic procedure and GC/MS analysis. These data confirm previous findings. A randomized, double-blind, placebo-controlled study will be initiated, in order to prove our hypothesis in a larger population.

2 METABOLIC ASSESMENT OF GRADUAL DEVELOPMENT OF MODERATE EXPERIMENTAL COLITIS IN IL-10 DEFICIENT MICE

Martin, Francois-Pierre; Rezzi, Serge; Montoliou Roura, Ivan; Philippe, David; Tornier, Lionel; Messlik, Anja; Hölzlwimmer, Gabriele; Baur, Pia; Quintanilla-Fend, Leticia; Loh, Gunnar; Blaut, Michael; Blum, Stephanie; Kochhar, Sunil; Haller, Dirk;

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Evidence has linked genetic predisposition and environmental exposures to the worldwide pandemic of inflammatory bowel diseases (IBD), but underlying biochemical events remain largely undefined. We studied the gradual development of colitis in Interleukin 10 deficient mice using a combination of (i) histopathological analysis of intestinal sections, (ii) metabolic profiling of blood plasma and (iii) measurement of plasma inflammatory biomarkers. Data integration using chemometric tools, including Independent Component Analysis, provided a new strategy for measuring and mapping the metabolic effects associated with the development of intestinal inflammation at the age of 1, 8, 16 and 24 weeks. Chronic inflammation appeared at 8 weeks and onwards, and was associated with altered cecum and colon morphologies and increased inflammatory cell infiltration into the mucosa and the submucosa. Blood plasma profiles provided additional evidence of loss of energy homeostasis, impaired metabolism of lipoproteins and glycosylated proteins. In particular, IL-10 -/- mice were characterized by decreased levels of VLDL and increased concentrations of LDL and polyunsaturated fatty acids, which are related to the etiology of IBD. Moreover, higher levels of lactate, pyruvate, citrate and lowered glucose suggested increased fatty acid oxidation and glycolysis, whilst higher levels of free amino acids reflected muscle atrophy, breakdown of proteins and inter-conversions of amino acids to produce energy. These integrated system investigations demonstrate the potential of metabonomics for investigating the mechanistic basis of IBD, and it will provide novel avenues for management of IBD.

3 REGULATION OF GOBLET CELL DIFFERENTIATION FACTORS HATH1 AND KLF4 IN COLONIC CELLS

Svetlana Becker, Julia Beisner , Michael Gersemann, Maureen Koslowski, Jan Wehkamp, Eduard F. Stange

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Backgrounds & Aims: The epithelium of the healthy colon is continuously covered by a mucus layer protecting it from luminal microbiota. The mucus is mainly formed by mucins that are secreted from goblet cells. The differentiation of intestinal progenitor cells into goblet cells is regulated by transcription factors Hath1 and KLF4 as well as components of Notch and Wnt pathways. Ulcerative colitis (UC) is associated with a reduced mucus layer and a defective goblet cell Hath1 and KLF4 induction (Gersemann et al., Differentiation 2009). The aim of the present study was to elucidate the regulation of Hath1 and KLF4 in colonic inflammation.

Methods: Experiments with a goblet cell lineage using LS174T colon adenocarcinoma cell line were performed. Cells were incubated with IL-1β, IL-4, IL-13, TNFα, IL-22 and killed *E. coli* K12 for various periods of time. Unstimulated cells were used as a negative control. The expression of Hath1, KLF4, mucins Muc1 and Muc2 as well as Hes1 genes was determined by real-time PCR.

Results: Hath1 and Hes1 expression was significantly induced by the interleukins IL-4 and IL-13, without affecting investigated mucins and KLF4. TNFα as well as IL-22 treatment resulted in upregulation of the Muc1 expression. Most importantly, Hes1 mRNA level was decreased after stimulation with killed *E. coli* K12 whereas Hath1 and KLF4 were unaffected.

Conclusion: Various cytokines affect goblet cell differentiation factors and a mucin (MUC1). The luminal microflora and goblet cells could be linked through the Notch pathway resulting in enhanced goblet cell differentiation.

4 THE TYPE I INTERFERON INDUCED GENE IFIT-2 COUNTERACTS DSS COLITIS

Susanne Berchtold, Alexandra Siegfried, Birgit Manncke, Ingo B. Autenrieth, Erwin Bohn

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Host defense against bacterial infections (*Y. enterocolitica*), Mouse infection models

Interferon-induced tetratricopeptide repeat protein (IFIT)-2 is a type I interferon induced gene which we found to be highly expressed during *Y. enterocolitica* infection as well as in a mouse model for IBD. The biological functions of IFIT-2 are so far elusive. It is only suggested that IFIT-2 may interact with the translation initiation factor eIF3c and may affect protein translation and that hIFIT-2 may influence migration of epithelial tumor cells. We demonstrate that forced IFIT-2 expression in RAW264.7 macrophages inhibits LPS induced TNF-α secretion which is probably due to posttranscriptional inhibition of TNF-α. involving the 3´UTR of this gene. These data suggest that IFIT-2 could be involved in the negative regulation of hyperinflammation. In line with these data, IFIT-2 knockout mice are more susceptible to colitis development after DSS treatment.

5 FORGOTTEN CULTIVABLE BUGS: IDENTITY AND FUNCTIONS OF NOVEL MOUSE INTESTINAL BACTERIA

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The fact that many mammalian intestinal bacteria are difficult to culture has favored the use of microbial molecular tools. Advances in molecular techniques have indeed led to crucial findings in microbial ecology. However, culture-based studies of yet unidentified bacteria give the opportunity to gain access to novel functions. The present work deals with the isolation of bacteria from mouse intestinal samples, focusing on the TNFdeltaARE mouse model of chronic ileitis. Eight bacterial strains were isolated from the ileal mucosa of a TNFdeltaARE mouse on mucin-containing agar, hinting at possible close interactions with intestinal epithelial cells (IEC). One isolate belonging to the family *Coriobacteriaceae*, resistant to ciprofloxacin, was a novel bacterium and was named *Enterorhabdus mucosicola*. Interestingly, the new species catalyzed the conversion of the isoflavone daidzein to equol, the function of which with respect to modulation of immune responses remains to be determined. Of note, the occurrence of *Coriobacteriaceae* in relation to inflammatory bowel diseases (IBD) has been recently reported by other groups. Since molecular chaperones may play important roles in IBD, we hypothesized that surface eukaryotic-chaperone-like structures from gut bacteria influence IEC functions. *E. mucosicola*, among six other strains including two novel *Coriobacteriaceae* and one novel *Streptococcus* sp., was isolated from the cecum of a 25-week-old TNFdeltaARE mouse using DYNAL magnetic beads coated with mouse anti-Grp-78 antibodies. Taxonomic and functional description of the isolates is underway, particularly concerning their involvement in immune and cell stress responses.

6 DRAMATIC DAMAGING EFFECTS OF *BACILLUS CEREUS* ON BARRIER FUNCTION OF INTESTINAL EPITHELIAL CELLS

Doll Viktoria, Ehling-Schulz Monika, Vogelmann Roger

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Scientific Interests: epithelial barrier function, gastroenteritis, Bacillus virulence factors
Modelsystems*:* *in vitro* polarized intestinal epithelial cells (IEC) (PTK6 cells), *Bacillus cereus* infection

The endospore forming bacterium *Bacillus cereus* has been increasingly associated with foodborne gastrointestinal diseases. *B. cereus* virulence is multifactorial due to several *B. cereus* toxins causing clinical symptoms such as emesis and diarrhea. We are interested in studying the importance of direct host-pathogen interaction in the intestinal tract. However, *B. cereus* infection has a dramatic effect on epithelial cell survival *in vitro*. In an *in vitro* model, we infected polarized colon epithelial cells (PTK6 cells) with 15 different *B. cereus* strains in mid-exponential growth phase using an MOI of 1. Eleven strains caused immediate cell detachment and cell death within 2 to 2.5 hrs after begin of infection. To our surprise this included two probiotic *B. cereus* strains, which equally to disease associated strains caused severe epithelial cell damage. Most of the tested strains express the pore-forming non-haemolytic enterotoxin (Nhe), which is associated with diarrheal disease. In order to test the role of Nhe toxin for IEC survival directly, we infected IEC with an *nheBC* deletion mutant (*B. cereus* strain NVH1173 kindly provided by Simon P. Hardy). This resulted in delayed detachment (3h) compared to wildtype (2h). The virulence regulator PlcR, which regulates almost the entire bacterial secretom of *B. cereus*, controls also Nhe expression. A PlcR deletion mutant did not cause any cell damage of IEC indicating that PlcR regulates bacterial virulence factors in addition to Nhe causing epithelial cell damage. Future experiments will focus on identifying these factors.

7 MOLECULAR DETECTION OF *ENTEROCOCCUS FAECALIS* SYMBIOFLOR 1 FOLLOWING ORAL INGESTION

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Scientific Interests / Modelsystems: Molecular biology, cellular microbiology, immunology, genomics and metagenomics, bioinformatics, tissue culture system, mouse infection model, *Galleria mellonella* infection model

Enterococci are members of the natural microbiota of animal and human intestinal tracts and are capable of causing opportunistic infections. They are also used as starter cultures in the food industry as well as in health supplements and probiotics by the pharmaceutical industry. We performed gapped-genome sequencing of the probiotic strain E. faecalis Symbioflor 1 using the Sanger sequencing technology and presented initial results derived from comparative genome analysis with that of the previously sequenced pathogenic clinical isolate E. faecalis V583. Since Enterococci are commensals we used information derived from the genome sequence to design a panel of PCR probes that were specific for the presence of Symbioflor 1 strain. A panel of 35 PCR-based probes was first used for validation on a random set of 110 enterococcal strains isolated from healthy and diseased individuals. Six of the PCR-based probes were used to detect for the presence of the Symbioflor 1 strain in serial samples of stools from probands prior to and following ingestion of the probiotic. The probiotic strain was detected after 2-3 days following ingestion and persisted for between 3–7 days after the last dose taken. Our data provide first information on the persistence of the probiotic Symbioflor 1 following oral ingestion.

8 ADHESION OF AN ANTI-INFLAMMATORY *B. BIFIDUM* STRAIN TO INTESTINAL EPITHELIAL CELLS IS MEDIATED BY PROTEINACEOUS CELL WALL COMPONENTS

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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.

We analysed the adhesive structures of *B. bifidum* S17 a strain that showed excellent adhesion to IECs in previous studies as well as potent inhibition of LPS-induced NF-κB activation. For this purpose, we established a method to obtain clean cell wall, membrane and cytoplasmic fractions of bifidobacteria. We were able to show, that in particular the cell wall fraction inhibits adhesion of whole cells of *B. bifidum* S17 to differentiated monolayers of IECs. To test whether the structures responsible for adhesion of *B. bifidum* S17 are cell surface proteins *B. bifidum* S17 was treated with pronase, lipase, and periodate and cell wall fractions were prepared after the treatment. Several bands are absent in the cell wall fraction from bacteria that were treated with pronase in comparison to the cell wall fraction from the untreated *B. bifidum* S17. Furthermore, treatment of *B. bifidum* S17 with pronase significantly decreased adhesion to IECs whereas treatment with lipase and periodate did not show any effects on adhesion. This indicates that proteinaceous cell surface components are involved in adhesion of *B. bifidum* to IECs.

9 VSL#3-DERIVED *L. CASEI* INDUCES POST-TRANSLATIONAL DEGRADATION OF IP-10 PROTEIN IN INTESTINAL EPITHELIAL CELLS: IMPACT ON CHRONIC INFLAMMATION

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Background. VSL#3 is a clinically relevant probiotic mixture in the context of inflammatory bowel diseases (IBD) but the underlying mechanisms are unclear. The aim of the study was to reveal bacterial strain-specific anti-inflammatory effects of VSL#3 on intestinal epithelial cells (IEC) and to analyse the protective potential of VSL#3 in the context of ileal inflammation.

Results. VSL#3 selectively inhibited TNF-induced secretion of the T-cell chemokine interferon-inducible protein (IP-10) in Mode-K cells. *Lactobacillus casei* (*L. casei*) cell surface as well as secreted proteins were identified as the underlying active components of VSL#3. *L. casei* failed to block TNF-induced IP-10 promoter activity and IP-10 gene transcription whereas IP-10 secretion and IP-10-mediated T-cell transmigration was inhibited. Kinetic studies, pulse-chase experiments and brefeldin A-induced blockade of the export machinery showed that *L. casei* did not impair initial IP-10 production but decreased intracellular IP-10 protein stability as a result of blocked IP-10 secretion. The inhibition of vesicular trafficking by 3-methyladenine inhibited IP-10 but not IL-6 expression, mimicking the inhibitory effects of *L. casei*. These findings suggest that *L. casei* impairs vesicular pathways important for the secretion of IP-10, followed by subsequent degradation of the proinflammatory chemokine. Feeding studies in $TNF^{\Delta ARE}$ and $IL\text{-}10^{-/-}$ mice revealed protective effects of VSL#3 on the development of cecal but not on ileal or colonic inflammation. Consistent with reduced tissue pathology in $IL\text{-}10^{-/-}$ mice, IP-10 protein expression was reduced in primary cecal epithelial cells.

Conclusion. We demonstrate intestinal segment specific effects of probiotic intervention correlating with IP-10 protein expression in the native epithelium. *L. casei*-induced post-translational degradation of IP-10 in IEC was found to be a new probiotic mechanism contributing to the anti-inflammatory effect of VSL#3.

10 TREATING PATIENTS WITH DYSBIOSIS- PRACTICAL CONSEQUENCES

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At the moment, scientifical research performs more and more studies about the importance of the human intestinal flora for a healthy organism. All the details of the host/ guest- interaction are astonishing, highlighting the importance of the connection that has developed over hundred thousands of years. Probiotics, especially lactic acid bacteria, are protecting against infections with mechanical (mucus), biological (lactoferrin) and immunological weapons (cytokines). A well balanced bacterial flora establishes an optimal barrier- function of the intestine, optimal resorption of nutritional substances, and a well- balanced local and global immune system. Scientists also speculate about the impact of pro- and dysbiotics on allergic diseases. This abstract exposes observations a practitioner has made in about 10 years work with patients. The most important symptoms dysbiosis-patients complain of are of gastrointestinal kind, as esophagitis, bloating, diarrea, obstipation and even hemorrhoids. Many patients have multiple symptoms: a reduced immune system, fatigue, muscle- and joint pain, headaches, skin diseases, allergies and so on. Frequently, patients report food intolerances (FI) lying behind their symptoms. F I is often the consequence of intestinal dysbiosis, and again the trigger for inflammatory reactions in the mucosa. The cytokines resulting of this inflammation can reach any organ by the blood stream, and hereby be the origin of “non bacterial inflammation”, as arthritis or inflammatory bowel disease. The treatment of FI consists of food testing (IgG4- panel for example), probiotic treatment and restoration of the mucosal barrier. After successful treatment, the most patients again tolerate all foods.

11 ROLE OF GUT MICROBIOTA IN ENERGY METABOLISM AND OBESITY DEVELOPEMENT IN MICE

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Intestinal bacteria affect the host energy harvest from the diet. This conclusion has been drawn from the observation that germfree in contrast to conventional mice were protected from diet-induced obesity. In addition, changes in the proportion of the two major intestinal bacterial phyla (Bacteroidetes and Firmicutes) have been reported in obese humans and in animal models for obesity. Proposed mechanisms of a bacterial contribution to obesity include a higher efficacy of polysaccharide utilization, a higher *de novo* hepatic lipogenesis and a reduced expression of the fasting-induced adipose factor (Fiaf) in conventional compared to germfree mice. We were interested in identifying bacterial species influencing the host energy metabolism and dietary factors contributing to the observed effects. Germfree and conventional mice were fed either a low fat (LF) or a high fat (HF) diet. Germfree mice fed the HF diet had a higher energy uptake and a lower energy expenditure than the conventional controls resulting in a significantly higher body weight and body fat content. When germfree and conventional mice were fed a HF diet with the same macronutrient proportions but differing in carbohydrate and fatty acid composition, germfree mice gained less body weight than the conventional animals. We detected higher fiaf mRNA levels in the intestinal mucosa of the germfree animals but failed to demonstrate elevated Fiaf protein levels in the mucosa or the plasma. Quantification of gut bacteria using the oligonucleotide probes Erec482 (Firmicutes), Lab158 (Firmicutes) and Bac303 (Bacteroides) revealed that the most dramatic changes in microbiota composition were induced by changing the diets from a standard chow to the purified experimental diets. In mice fed the standard chow, 66 % of the total bacteria were detected by the three probes but the percentage significantly decreased to 31 % and 18 % in animals fed the LF diet and HF diet, respectively. Sequence analysis and taxonomic assignment of 16S rRNA gene fragments revealed that *Erysipelotrichaceae* strongly increased in response to the HF diet. Nothing is known about the role of this bacterial family in the intestine. We conclude that not only host-bacteria interactions influence energy metabolism but that the type of dietary carbohydrates or fat can modify the interplay between host and bacteria.

12 ADHESION, INVASION AND PROLIFERATION OF THE FACULTATIVE PATHOGEN *HELICOBACTER HEPATICUS* IN EPITHELIAL CELLS

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Scientific Interests: IBD, epithelial barrier function, *Helicobacter hepaticus*
Modelsystems: in vitro polarized IEC (PTK 6), *H. hepaticus* infection

Inflammatory Bowel Disease (IBD) is characterized by chronic inflammation caused in part by leaky intestinal barriers allowing facultative pathogens to interact with the host immune system. In IBD and IBD mouse models increased attachment and invasion of bacteria to intestinal epithelial cells (IEC) can be observed. We were interested if otherwise non-invasive facultative pathogens adhere and invade IEC when the epithelial barrier is defective. Therefore, we used an in vitro model for polarized mouse colon epithelial cells (PTK6 cells) and infected with *H. hepaticus*.

We are able to show, that adhesion of bacteria to epithelial cells depends on barrier function. Almost no bacteria adhered to the apical surface, when polarized IEC were infected for 18 h with intact barriers, compared to133 bacteria per 100 cells, when infected with an open barrier. Adhesion was followed by invasion (70 bacteria/100 cells after 18 h only with barrier defect) and electron- and immunofluorescence microscopy data suggest that *H. hepaticus* localizes to the lysosome before entering the cytoplasm of IEC. In gentamycin protection assays we were able to show intracellular survival and growth of *H. hepaticus*. During treatment of IEC with gentamycin as antibiotic at a lethal dose, intracellular bacteria increased from 24 bacteria/100 cells after 2 h to over 500 bacteria/100 cells after 48 h. After removal of gentamycin, bacteria were able to repopulate cell culture media only in presence of IEC. Inhibitor experiments revealed that the endocytosis mechanism used by bacteria is caveolin-mediated. During infection without inhibitors, or with inhibitors for clathrin-mediated endocytosis 200 bacteria/100 cells internalized. When inhibiting caveolin-mediated endocytosis, 100 bacteria/100 cells invaded. Our data suggest, that *H. hepaticus* adheres to and invades IEC once the epithelial barrier is broken. Invasion is caveolin-mediated, which maybe is a target to prevent *H. hepaticus* induced chronic inflammation.

13 THE IMMUNOMODULATORY PROPERTIES OF DIFFERENT PROBIOTIC STRAINS AND SPECIES

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Previous studies have shown that there is a correlation between the anti-inflammatory cytokine profiles of probiotic-stimulated human PBMC and their ability to attenuate colitis in a mouse model. However, there is a need for more understanding of the bacterial factors and cellular receptors that determine the immunomodulatory properties of different probiotic and commensal bacteria. For different commensal/probiotic species and strains we measured cytokine profiles in co-culture with human PBMCs and their capacity to activate TLR2. Additionally, the effects of probiotics on dendritic cell (DC) maturation and function were investigated using human monocyte derived DCs. Probiotic species differ widely in their ability to stimulate the secretion of IL-10 and IL-12 in co-culture with human PBMC; variation is also seen at the strain level. Probiotics also differ widely in their capacity to activate human TLR2. Activation of TLR2 was shown to be an important factor influencing the patterns of cytokines induced by probiotics and their ability to activate DCs at low bacteria to cell ratios.

14 *LACTOBACILLUS REUTERI* 100-23 TRANSIENTLY ACTIVATES INTESTINAL EPITHELIAL CELLS OF MICE THAT HAVE A COMPLEX MICROBIOTA DUNRING EARLY STAGES OF COLONIZATION

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Monoassociation of germ-free animals with colitogenic or probiotic bacterial strains trigger intestinal epithelial cell (IEC) activation and host-derived feedback mechanisms. To characterize the impact of a single nonpathogenic bacterial strain on the intestinal epithelium in the presence of an established microbiota, we inoculated reconstituted *Lacotobacillus*-free (RLF) mice at 8 wk of age with *Lactobacillus reuteri* 100-23. Primary IEC from the small intestine of *L. reuteri*-inoculated and control RLF mice were isolated 2, 6, and 21 d after inoculation followed by gene expression analysis (real-time PCR; Affymetrix microarrays) as well as 2-dimensional-gel electrophoreses (2D SDS-PAGE) and peptide mass fingerprinting via matrix-assisted laser desorption/ionization time of flight MS. At d 6, gene expression of proinflammatory cytokines and chemokines including interleukin (IL)-1α, IL-6, interferon-γ-inducible protein 10, and macrophage inflammatory protein 2 was transiently induced, whereas gene expression levels of regulatory proteins A20 and Toll-interacting protein decreased. In addition, 8 target proteins with changes in the steady-state protein expression levels were identified at d 2 and 6 of *L. reuteri* colonization. Consistent with the absence of histopathology, *L. reuteri*-induced activation of primary IEC returned to control levels by d 21 after inoculation of RLF mice. The capability of *L. reuteri* 100-23 to directly trigger epithelial cell activation was confirmed in small IEC cultures using the murine cell line Mode-K. These results clearly indicate that the intestinal epithelium is reactive toward environmental changes induced by the commensal bacterial strain *L. reuteri* even in the presence of an already-established microbiota. The induction of transient IEC activation may help to maintain mucosal homeostasis.

15 DIETARY INTERVENTION WITH PROBIOTICS AFFECTS THE GENOTOXICITY OF FAECAL WATER IN AD PATIENTS

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The probiotic potential to reduce the production of toxic and carcinogenic metabolites by changing the composition and metabolic activity of the microbiota, decreasing colonic pH and/or suppressing specific enzyme activities in the colon is discussed.

An investigation was performed to study the effect of a probiotic mix consisting of *Lactobacillus paracasei* LPC37, *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 on the genotoxic activity of faecal water in healthy subjects and patients with atopic dermatitis (AD).

A placebo-controlled and cross-over study was conducted. The 15 healthy adults and the 15 patients with AD consumed 2 x 100 ml/d of a probiotic or a placebo drink for 8 weeks. A wash-out period of 2 weeks was interconnected before the intervention was crossed. Stool samples were collected at the end of each period and faecal water was isolated. HT29 human adenocarcinoma cells were incubated with faecal water and DNA damage was measured using the “comet assay” The concentrations of short-chain fatty acids were measured by gas chromatography.

In healthy subjects the tail intensity of faecal water, as indicator for the induction of DNA was not affected. However, in AD patients tail intensity was significantly decreased in the probiotic period compared to placebo (23.5 vs. 16.7%). The faecal concentrations of short-chain fatty acids remained unchanged in both groups, but faecal pH was significantly reduced in the probiotic period (7.0 vs. 6.6) in AD patients.

The results suggest that the probiotics lower the genotoxic burden of faecal water in AD patients. Despite the probiotic mix had no effect on the faecal concentrations of short-chain fatty acids, the decreased faecal pH in patients with AD indicates that other metabolic end products are affected.

16 COMMENSAL MICROFLORA AND RORγT ARE REQUIRED FOR THE DIFFERENTIATION OF MUCOSAL IL-22-PRODUCING NKP46$^+$ CELLS

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The mucosal immune system of the intestine is separated from a vast array of microbes by a single layer of epithelial cells. Cues from the commensal microflora are needed to maintain epithelial homeostasis, but the molecular and cellular identities of these cues are unclear.

Recently, data became available identifying two distinct subsets of mucosal NKp46$^+$ lymphocytes based on the expression of the orphan transcription factor RORγt. In many ways, the RORγt$^-$ subset resembled "classical" NK cells in that it was developmentally dependent on IL-15 but not on RORγt and displayed NK cell function (e.g., cell-mediated cytotoxicity, IFN-γ production). In contrast, the RORγt$^+$ subset developed independent of IL-15 but required RORγt suggesting that this subset may be related to lymphoid tissue inducer (LTi) cells.

Here we provide evidence that signals from the commensal microflora contribute to the differentiation of a lymphocyte population coexpressing stimulatory natural killer cell receptors and the transcription factor RORγt that produced interleukin 22 (IL-22). The emergence of these IL-22-producing RORγt^{hi}NKp46$^+$NK1.1(int) cells depended on RORγt expression, which indicated that these cells may have been derived from lymphoid tissue-inducer cells. IL-22 released by these cells promoted the production of antimicrobial molecules important in the maintenance of mucosal homeostasis.

17 INVESTIGATIONS ON THE ANTIMUTAGENIC ACTIVITY OF THE PROBIOTIC *ESCHERICHIA COLI* STRAIN NISSLE 1917

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Background: Escherichia coli strain Nissle 1917 (EcN, Mutaflor) has been shown to be as effective as standard therapy with the anti-inflammatory substance mesalazine (5-aminosalicylic acid) for maintenance of remission in patients with ulcerative colitis (UC) (1). Mesalazine is thought to also reduce the elevated risk of UC patients for the development of colorectal carcinoma. In that respect, there are no clinical data for long-term use of EcN so far. Since it has been shown that some strains of the genera *Bifidobacterium* and *Lactobacillus* exhibit antimutagenic/anticarcinogenic activity (2), we investigated whether this is also the case for the EcN strain.

Materials and Methods: Two different mutagenicity tests have been employed: the classical Ames test (3) with mutants of *Salmonella* and *Escherichia coli* and the Comet assay with Caco-2 epithelial cells (4). As mutagenic substances 4-nitroquinoline-1-oxide (NQO), benzo(a)pyrene (B(a)P), and H_2O_2 have been used. NQO was tested in both mutagenicity assays after coincubation with either live EcN, heat-killed EcN or cell-free spent supernatants of EcN cultures. B(a)P was examined in the Ames test, and H_2O_2 in the Comet assay. After separation from bacterial cells by sterile filtration, the coincubation mixtures were tested for residual mutagenic activity, and compared to untreated mutagen solutions.

Results: EcN itself did not show any mutagenic activity, but led to a loss of mutagenicity of NQO, B(a)P, and H_2O_2 in a dose-dependent manner. The antimutagenic effect could only be shown with live EcN bacteria. Heat-killed EcN and spent supernatants had no antimutagenic activity. In the case of NQO, the antimutagenic action of EcN led to a shift in the absorption spectrum of NQO, as revealed by UV/VIS spectroscopy, suggesting biochemical transformation of NQO.

Conclusions: As live EcN bacteria display antimutagenic action against the mutagenic substances NQO, B(a)P, and H_2O_2, it may be speculated that UC patients might also derive benefit from long-term treatment with EcN.

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18 BACTERIAL PROTEASES CONTRIBIUTE TO THE DEVELOPMENT OF CHRONIC INTESTINAL INFLAMMATION THROUGH THE IMPAIRMENT OF EPITHELIAL BARRIER FUNCTIONS

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Background. The activation of endogenous matrix metalloproteinases (MMPs) plays an important role in the pathogenesis of chronic intestinal inflammation. The approach of our study was to investigate whether bacteria-derived proteases in the gut lumen also affect the development of experimental colitis in gnotobiotic Interleukin 10 deficient (IL-10-/-) mice.

Results. Monoassociation experiments of wild type (WT) and IL-10-/- mice (129SvEv) with *Enterococcus faecalis* (*E. faecalis*) strain OG1RF revealed increased mRNA expression levels of the bacterial gelatinase E (GelE) under conditions of experimental colitis. To further characterize the role of this bacterial protease in the pathogenesis of chronic intestinal inflammation, we monoassociated WT and IL-10-/- mice for 15 weeks with *E. faecalis* strain OG1RF and isogenic mutant strains that lack GelE expression including TX5264 (gelE deletion) and TX5266 (fsrB deletion). Histopathological analysis revealed a significant reduction of distal and proximal colonic tissue pathology in the absence of bacterial GelE. To further investigate the impact of GelE on barrier function of intestinal epithelial cells, we performed transwell experiments with human (T84) and mouse (Ptk6) colonocytes. The transepithelial electrical resistance (TER) was measured to monitor the integrity of the cells. Apical stimulation of the transwell cultures with concentrated conditioned media (CM) of *E. faecalis* OG1RF reduced TER values by approximately 70%. In contrast, GelE deficient mutant strains (TX5264 and TX5266) did not affect barrier integrity of the epithelial cell cultures. Consistent with these results, purified proteolytically active GelE caused a dramatic decrease in TER values as well supporting the hypothesis that GelE impairs barrier integrity of the intestinal epithelium. In this context we identified the adherence junction proteins E-Cadherin and β-Catenin as potential targets of GelE.

Conclusion. We showed that the *E. faecalis*-derived gelatinase E contributes to the development of experimental colitis in IL-$10^{-/-}$ mice. In transwell cultures GelE led to the impairment of epithelial barrier integrity, suggesting that bacterial proteases contribute to the pathogenesis of intestinal inflammation.

19 INCREASE OF A SINGLE *ESCHERICHIA COLI* STRAIN IN THE GUT OF COLITIC MICE SEEMS TO BE MEDIATED BY THE ENHANCED EXPRESSION OF THE *NEUC* GENE

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Commensal bacteria play a role in the aetiology of inflammatory bowel diseases (IBD). High intestinal numbers of *Escherichia coli* in IBD patients suggest a role of this organism in the initiation or progression of chronic gut inflammation. In addition, some *E. coli* genotypes are more frequently detected in IBD patients than others. We aimed to find out whether gut inflammation in an IBD mouse model is associated with a particular *E. coli* strain.

Intestinal contents and tissue material were taken from 1, 8, 16, and 24 week old Interleukin 10 deficient (IL-$10^{-/-}$) mice and the respective wild type animals. Caecal and colonic inflammation was observed in IL-$10^{-/-}$ animals from the eighth week of life, accompanied by a lower intestinal microbial diversity than in the respective wild type animals. Sequencing analysis of bacterial 16S rRNA genes revealed that IL-$10^{-/-}$ mice harbour a significantly higher proportion of sequences belonging to *Escherichia coli, Blautia producta* and *Entercoccus gallinarum* than healthy controls.

Moreover, culture based and molecular approaches showed that cell numbers of *E. coli* were significantly higher in IL-$10^{-/-}$ mice compared to the wild type animals and that all animals were colonized by one single *E. coli* strain. The strain was shown to have the O7:H7:K1 serotype and to belong to the virulence-associated phylogenetic group B2. A high number of virulence and fitness associated genes was detected in the strain's genome. Real time PCR analysis of *E. coli* virulence genes, using bacterial RNA isolated from caecal contents of 16 week old IL-$10^{-/-}$ and wild type mice, revealed furthermore that the *neuC* gene encoding the K1 capsule of the O7:H7:K1 strain is 6 fold upregulated under inflammatory conditions. This finding suggests that the K1 capsule is possibly involved in the adaptation of the isolated *E. coli* strain to inflammatory conditions of the murine gut.

20 INVESTIGATING THE PROBIOTIC POTENTIAL OF *ESCHERICHIA COLI* NISSLE 1917 BY USING *IN VITRO* ADHERENCE ASSAYS

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The probiotic strain *Escherichia coli* Nissle 1917 (EcN) is widely used for the treatment of several gastrointestinal disorders such as infectious diarrhea or remission maintenance in patients with ulcerative colitis. However, despite the longstanding experience with this strain, the molecular mechanisms responsible for the probiotic character of EcN are not completely understood.

In order to establish a test system suitable for evaluating the probiotic potential of EcN, we employed enteropathogenic *E. coli* (EPEC) or the mouse-pathogen *Citrobacter rodentium* in *in vitro* adherence assays. Both enteric pathogens attach to luminal surfaces of host intestinal epithelial cells and efface localized regions of microvilli. Adherence assays were performed in a two step protocol. Firstly, human intestinal epithelial Caco-2 or Lovo cells were co-incubated with EcN. Following a two hour incubation step, EcN was removed by vigorous washing and cells were infected with EPEC or *C. rodentium*. Pre-treatment with EcN significantly reduced adherence of both diarrheagenic strains. In this system we will now be able to evaluate adherence inhibition of enteropathogens, which we consider a probiotic trait of EcN. On the one hand, we can test various isogenic mutants of EcN with this assay and on the other hand, the use of *C. rodentium* opens the way to perform *in vivo* experiments in a mouse model.

Furthermore, we tested the behavior of uropathogenic *E. coli* CFT073. Comparing the genome sequences of EcN and *E. coli* CFT073 revealed that both strains are highly similar and pre-treatment with *E. coli* CFT073 leads to an almost complete prevention of EPEC adherence *in vitro*. We are now investigating the molecular mechanisms of this effect by genome comparison and construction of scarless isogenic deletion mutants.

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