5th Seeon Conference

Microbiota, Probiota and Host

Mikrobiota, Probiota und Wirt

15.- 17. June 2012

Conference Center

Monastery Seeon / Chiemsee

For more information:

DGHM@wzw.tum.de

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

June 15, 2012

Dear Participant,

On behalf of the German Society of Hygiene and Microbiology (DGHM) and the Organizing Committee, welcome to the 5th Seeon Conference “Microbiota, Probiota and Host”!

The dramatic increase of chronic inflammatory and degenerative diseases particularly in the industrialized world implies a dynamic interaction of disease susceptible genomes with an enormously complex environment. Nutrition-related factors together with components of mucosa-associated microbial ecosystems especially in the gastrointestinal system emerged as prime environmental triggers for the development and modification of metabolically-driven and inflammation-mediated pathologies.

The past few years the newly founded DGHM section “Microbiota, Probiota and Host” has established a visible community of talented young and senior scientists across various disciplines including basic science, genetics, and clinicial disciplines such as gastroenterology, medical microbiology and immunology, as well as nutritional medicine. The “Seeon Conference” has become a known platform to critically discuss the role of microbe-host interactions in health and disease sharing cutting-edge science and technologies. Basis mechanisms of the host’s microbiome are discussed at the interface of metabolic and immune functions aiming to be implemented in therapy and prevention of chronic inflammatory, atopic and metabolic diseases.

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

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PROGRAM Friday, June 15

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

17^{15} – 18^{00}	Keynote Lecture: **Christof R. Hauck**, Cell Biology, University Konstanz ***Cellular adhesion molecules as targets for pathogens and commensals***

18^{15}	Dinner

19^{15}	DGHM Section Meeting

GUT MICROBIOME AND HOST

19^{45}– 21^{15} Chair: D. Haller, Biofunctionality, TU Munich

K. Peter, Hematology and Oncology, University Hospital Regensburg
Intestinal dysbiosis in patients receiving allogeneic stem cell transplantation: Impact of antibiotic decontamination and GvHD

A. Woting, German Institute of Human Nutrition, Nuthetal
Role of Clostridium ramosum (Erysipelotrichaceae) in obesity development

S. Brugiroux, Max-von-Pettenkofer Institut, LMU Munich
Generation of gnotobiotic mice harboring a defined consortium of mouse intestinal microbiota

J. Wang, Max-Planck-Institute for Evolutionary Biology + University of Kiel
Host-intestinal microbiota coevolution in the house mice

C. A. Kolmeder, Department of Veterinary Biosciences, University of Helsinki
Integrated metaproteomic and phylogenetic analysis of the intestinal microbiome of healthy individuals

M. Ege, University Children's Hospital Munich
Exposure to environmental microorganisms and its inverse relation to childhood asthma

21^{15}	Drink at the Bar?

PROGRAM Saturday, June 16

08^{30} – 09^{15} Keynote Lecture: **Arlette Darfeuille-Michaud**, CBRV, Clermont-Ferrand, France
Adherent-invasive E. coli in Crohn's disease

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

PROBIOTIC MECHANISMS

09^{45} – 11^{15} Chair: G. Loh, German Institute of Human Nutrition, Nuthetal

S. Herbel, Institute of Microbiology and Epizootics, Freie Universität Berlin
Detection, identification and quantification of different probiotic Lactobacilli in food by using a multiplex qPCR method

J. Schützner, Institute for Microbiology and Biotechnology, Ulm University
Treatment with a B. bifidum strain ameliorates colitis in three mouse models of colitis

S. Rund, Institute of Molecular Infection Biology, University of Wuerzburg
Antagonistic effects of E. coli Nissle 1917 on various EHEC strains

L. Nedialkova, Max-von-Pettenkofer Institute, LMU Munich
The role of the host response in colicin-dependent E. coli –Salmonella competition in the gut

K. Hochwind, Research Unit Microbe-Plant Interactions, Helmholtz Zentrum München
Identification of D-tryptophan as immunologically active compound excreted by probiotic bacteria using immunological in vitro-test systems

P. Rausch, Max-Planck-Institute for Evolutionary Biology + University of Kiel
Global patterns of the standing and active human gut microbiome in health and inflammatory bowel diseases

11^{15} – 12^{00} Keynote Lecture: **Michael Bailey**, Comparative Immunology, Langford, U.K.
Manipulation of the immune system through microbiota using a translational animal model

PROGRAM Saturday, June 16

12^{00} - 14^{00}	Lunch and “Mozart in Seeon: Mini-Concert in the Chapel St. Nikolaus“

MECHANISMS OF INFLAMMATION

14^{00} – 15^{30} Chair: C. Riedel, Inst. for Microbiology + Biotechnology, Ulm University

S. Lipinski, Institute of Clinical Molecular Biology, CAU Kiel
Extracellular Cathepsin K is protective against chronic intestinal inflammation in mice and involved in host-microbe homeostasis

A. Steimle, Med. Microbiology + Hygiene, University Tübingen
Molecular mechanisms leading to semi-mature murine dendritic cells and their role in intestinal homeostasis

M. Weiher, Biofunctionality, TU Munich
Antibiotic treatment changes gut bacterial diversity and protects TNFdeltaARE mice from Crohn’s disease-like ileitis

A. Schmidt, Biofunctionality, TU Munich
Dietary factors in the regulation of Crohn’s disease-like ileitis

C. Manta, Dep. of Internal Medicine I + Department of Biotechnology, Ulm University
CX3CR1+ Macrophages induce the expansion of IL-22 producing CD4+RORγt+CD3- Innate Lymphocytes required for the control of a C. rodentium infection

K. Radulovic, Department of Internal Medicine I, Ulm University
The early activation marker CD69 regulates the intestinal inflammation by affecting migration of CD4 T cells and generation of Treg cells

15^{30} – 16^{00}	Coffee Break
16^{00} – 18^{00}	**Poster Slam** (2 minutes / 2 slides) **and Poster discussion** (J. Frick, Institute for Medical Microbiology + Hygiene, Tübingen)
18^{00} – 18^{45}	Keynote Lecture: **Antonio Molinaro**, Organic Chemistry and Biochemistry, University Naples, Italy ***Microbial cell surface glycoconjugates and elicitation of eukaryotic innate immunity***
18^{45}	Dinner
20^{30}	Bowling at the Bar

PROGRAM Sunday, June 17

08^{30} – 09^{15} Keynote Lecture: **Daniel Huson**, Algorithms in Bioinformatics, University Tübingen
Bioinformatics analysis of microbiome sequence data

09^{15} – 09^{45} Coffee Break

09^{45} – 10^{00} **Poster Award**

MECHANISMS OF INFECTIONS

10^{00} – 11^{30} Chair: B. Stecher, Max-von-Pettenkofer Institut, LMU Munich

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

M.M. Heimesaat, Inst. for Microbiology + Hygiene, Charité – University Med. Berlin
Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

A.-K.Claes, Inst. for Experimental Med., University of Kiel / Research Center Borstel
Salmonella-infection leads to exacerbated colitis in Nod2-deficient mice

E. Heupel, Institute of Microbiology and Biotechnology, University of Ulm
Citrobacter rodentium-induced colitis in mice is alleviated by pre-treatment with Bifidobacterium bifidum S17

N. Waldschmitt, Biofunctionality, TU Munich
Association of C/EBP homologous protein in intestinal epithelial cells with the development of chronic inflammation: a transgenic approach

D. Schultz, Inst. for Experimental Med., University of Kiel / Research Center Borstel
NLRP6-deficiency results in a higher susceptibility to Salmonella enterica serovar Typhimurium infection in mice

11^{30} Lunch and Departure

PROGRAM
Friday,
June 15

CELLULAR ADHESION MOLECULES AS TARGETS FOR PATHOGENS AND COMMENSALS

C.R. Hauck

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Cell adhesion molecules, including integrins, cadherins and members of the immunoglobulin-superfamily (IgCAMs) are essential components of multicellular organisms in the animal kingdom. They provide a reliable connection between isolated cells as well as between cells and the extracellular matrix. Thereby, they are a critical pre-requisite for the formation of tissues and organs.

Interestingly, cell adhesion molecules often serve as targets for viral and bacterial pathogens, which employ specific adhesive proteins (adhesins) to connect to surface structures of their eukaryotic hosts. In strong contrast to other abundant families of eukaryotic cell surface receptors, a disproportionately large group of microbes binds to various cell adhesion molecules. Indeed, integrins, cadherins as well as IgCAMs are common targets for exploitation by distinct bacterial adhesins. Not surprisingly, this initial encounter at the cellular level often is a critical determinant of pathogen-host specificity and constitutes a decisive point for the infection process as a whole.

The lecture will present current concepts of adhesin-mediated bacteria-host cell interaction by focussing on selected examples studied in my laboratory. In the last couple of years, the detailed analysis of adhesin-receptor interactions and the role of these adhesin-mediated processes has yielded fascinating insight not only in infection biology, but also has implications for our understanding of the co-evolution between microbes and their host organisms.

GUT MICROBIOME AND HOST

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Host-intestinal microbiota coevolution in the house mice

C. A. Kolmeder, Department of Veterinary Biosciences, University of Helsinki
Integrated metaproteomic and phylogenetic analysis of the intestinal microbiome of healthy individuals

M. Ege, University Children's Hospital Munich
Exposure to environmental microorganisms and its inverse relation to childhood asthma

INTESTINAL DYSBIOSIS IN PATIENTS RECEIVING ALLOGENEIC STEM CELL TRANSPLANTATION: IMPACT OF ANTIBIOTIC DECONTAMINATION AND GvHD

K. Peter[1], P. Butzhammer[2], K. Landfried[1], M. Kreutz[1], J. Koestler[3], A. Gessner[3], P. Oefner[2], E. Holler[1]

[1]Department of Hematology and Oncology, University Hospital Regensburg
[2]Institute of functional genomics, University Hospital Regensburg
[3]Department of Medical Microbiology and Hygiene, University Hospital Regensburg

Recent studies identifying SNPs of the bacterial cell wall ligand NOD2/CARD15 as risk factors and Reg3a, an antimicrobial peptide, as an indicator of intestinal GvHD suggested involvement of changes in the intestinal microbiome in the process of GvHD. To address the development of the intestinal microbiome in the setting of human allogeneic stem cell transplantation, we performed a prospective study in 31 patients: Stool samples were collected prior to conditioning, at d 0, during the aplastic period and after engraftment until d 100 after SCT. During the aplastic phase and in the period of intestinal GvHD, patients received antibiotic decontamination using ciprofloxacine and metronidazole, which was stopped in asymptomatic patients after engraftment. 16s rRNA sequencing was performed to identify major changes in the microbial phyllae, and microbiome shifts were correlated with the use of decontamination and occurrence of GvHD. While samples obtained at admission showed a microbial distribution which was comparable to healthy donors, major changes were observed starting from day 0: Specifically, the proportion of enterococci rose from almost 0% in donors pretransplant and 8% (+/-6%) in patients pretransplant to 27% (+/-5%) in patients receiving decontamination but not developing GvHD and further increased in those with subsequent intestinal GvHD to 58% (+/-6%) (p 0.004 vs. other groups). In parallel, the proportion of other firmicutes including lactobacilli dropped. In long term patients without GvHD and decontamination, microbiom patterns returned to pretransplant distribution, whereas patients with GvHD demonstrated a continuous high proportion of enterococci. Multivariate analysis suggested that both, decontamination but also development of intestinal GvHD independently contributed to dysbiosis as indicated by the enterococcal shift. Our data indicate that antibiotic decontamination favors enterococcal overgrowth which is more pronounced in patients with development of GvHD. Altered, mainly diminished production of antimicrobial peptides by destruction of Paneth cells as suggested by recent experimental data may explain this new effect in intestinal GvHD.

ROLE OF *CLOSTRIDIUM RAMOSUM* (ERYSIPELOTRICHACEAE) IN OBESITY DEVELOPMENT

A. Woting, G. Loh, M. Blaut

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

Background: In mice fed a high fat diet bacterial species belonging to the family Erysipelotrichaceae proliferate. Members of this family possibly promote the development of obesity and its related pathologies. We used a mouse model with a defined microbial status to investigate the role of *C. ramosum*, a member of the Erysipelotrichaceae, in the development of obesity and to identify adipogenic factors.

Methods: We established a mouse model with a simplified human microbiota (SIHUMI) consisting of eight bacterial species: *Anaerostipes caccae, Bacteroides thetaiotaomicron, Bifidobacterium longum, Blautia producta, Clostridium ramosum, Clostridium butyricum, Escherichia coli* and *Lactobacillus plantarum.* The use of SIHUMI mice containing only seven bacterial species without *C. ramosum* (SIHUMI-Cra), allows us to clarify the effect of *C. ramosum* on the development of symptoms of the Metabolic Syndrome.

Results: SIHUMI-Cra mice fed a high fat diet (HFD) or a low fat diet (LFD) for four weeks showed no differences in body weight gain and body fat mass. SIHUMI-Cra mice fed the HFD gained significantly less body weight and body fat than SIHUMI mice fed the HFD. The mice did not differ in their energy intake or the energy they digested. SIHUMI-Cra mice had less fecal acetic acid and propionic acid concentrations than SIHUMI mice. On the other hand the cecal and colonic acid concentrations did not differ between the mice. Mice monoassociated with *C. ramosum* fed the HFD or the LFD had a similar body weight and body fat development as SIHUMI mice. These mice will be used to identify adipogenic factors of *C. ramosum.*

GENERATION OF GNOTOBIOTIC MICE HARBORING A DEFINED CONSORTIUM OF MOUSE INTESTINAL MICROBIOTA

S. Brugiroux[1], Y. Lötscher[2], M. Diehl[1] and B. Stecher[1]*

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

[2]*Institute of Microbiology, ETH Zürich; current address: Inst. f. Agrarwissenschaften ETH Zürich, Switzerland,*

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In 1996, Rodney D. Berg estimated that the human gastrointestinal (GI) tract harbors 10 times more viable bacteria than cells in the human body (Berg 1996). Recent studies in humans and mice have established that diversity of intestinal microbiota is immense, harboring between 200 to 1,000 different species, the majority being strictly anaerobic. The high complexity of the intestinal ecosystem precludes investigating the contribution of individual strains to host-bacterial interactions as well as studying their individual role within the intestinal ecosystem. To this end, gnotobiotic mice harboring a defined selection of strains have proven highly useful. The majority of current gnotobiotic mouse models are based on using a selection of human isolates. The “Altered Schaedler Flora”, a collection of 8 mouse isolates which had been widely used in the past, is not any more available to the public. For this reason we isolated a collection of bacterial strains, the Mouse Minimal Microbiota (MMM), abundant in the mouse intestinal tract. The MMM so far comprises 16 isolates derived from 6 eubacterial phyla (Firmicutes, Bacteroidetes, Deferribacteries, Actinobacteria, Verrucomicrobia and Proteobacteria). We established a system using frozen mixtures of these isolates which can be used to reproducibly colonize gnotobiotic mice with this MMM. We adapted molecular tools to detect these strains in this mouse model (FISH, qPCR, 454-sequencing, draft genome sequencing). We expect that this collection of well characterized strains will be a useful tool for the scientific community in the future in order to address specific aspects of bacteria-host interaction and understand the role of single species in a complex microbial consortium.

*Berg, R. D. (1996). "The indigenous gastrointestinal microflora." Trends Microbiol **4**(11): 430-435.*

HOST-INTESTINAL MICROBIOTA COEVOLUTION IN THE HOUSE MICE

J. Wang , J. Baines

Max-Planck-Institute for Evolutionary Biology and Christian-Albrechts- University of Kiel

The mammalian intestinal microbiota are not obligatory symbionts, but do play numerous important roles in host biology. To shed light on the magnitude and consequences of coevolution between hosts and their intestinal microbial communities on a recent (~500,000 year) timescale, we are studying two subspecies of house mice (Mus musculus musculus and M. m. domesticus) which form a naturally occuring hybrid zone through Central Europe. We found that both in nature and controlled lab environments, the intestinal microbiota of hybrid mice display several characteristics distinct from the patterns observed in either parental species including significant differences in both alpha and beta diversity as well as changes in the abundance of individual bacterial taxa. Given relationship between the diversity of intestinal communities and susceptibility to enteric pathogens, we suggest these traits may lower the fitness of hybrid mice.

To conduct the investigation further we apply genome mapping approaches, using an F2 cross between two inbred mouse strains representative of the two subspecies M. m. musculus and M. m. domesticus (PWD and WSB) to perform QTL mapping, in order to identify the regulating gene for whole community or certain individual bacterial taxa and identify potential incompatible pairs of genetic loci following the Dobzhansky-Muller model. Complimentary to that, we are also cultivating one commensal genus of bacteria, which showed in previous QTL studies and certain gene knockout studies. Using comparative genomics approaches we would gather more insights into the co-evolution/divergence of host and microbes, and explain the interaction between host genome (genes) with bacteria genome.

INTEGRATED METAPROTEOMIC AND PHYLOGENETIC ANALYSIS OF THE INTESTINAL MICROBIOME OF HEALTHY INDIVIDUALS

C.A. Kolmeder[1], J. Salojärvi[1], A. Palva[1], A. Salonen[1], W.M. de Vos[1,2]

[1]*Department of Veterinary Biosciences, University of Helsinki, P.O. Box 66, FIN-00014 Helsinki, Finland*

[2]*Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands*

Phylogenetic approaches and metagenomic sequencing efforts revealed the species variety and enormous genetic content of our gut symbionts. To study the microbial actions *in vivo,* we developed a metaproteomic approach, i.e. analyzing the proteins derived from faecal material. To define a baseline of the metaproteome in healthy individuals, and to have grounds to compare it to diseased, we determined the metaproteome of 16 healthy individuals. Faecal samples were taken three times within 12 weeks. We applied a screening method using protein extraction by bead beating of crude faecal material, 1D PAGE protein fractionation, LC-MSMS analysis, and an in-house human intestinal database completed by scripts in languages Python and R for protein identification. Additionally, we used a phylogenetic microarray to describe the composition of the studied microbiotas. We could confirm and extend the subject-specific clustering of the intestinal metaproteome that we earlier described in a proof-of-principal study [Kolmeder CA et al. (2012) PLoS One 7(1): e29913.]. This data set now allows us to determine the shared and individual-specific intestinal functions, and to link specific proteins with microbial species.

EXPOSURE TO ENVIRONMENTAL MICROORGANISMS AND ITS INVERSE RELATION TO CHILDHOOD ASTHMA

M. Ege[1], M. Mayer[2], A.-C. Normand[3], J. Genuneit[4], W. OCM Cookson[5], C. Braun-Fahrländer[6,7], D. Heederik[8], R. Piarroux[9], E. von Mutius[1]

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Background: Children growing up in environments rich in microbial exposures such as traditional farms are protected from childhood asthma and atopy. In prior studies markers of microbial exposure such as endotoxin or muramic acid have been inversely related to these conditions.

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

Results: In both studies, farm children had lower prevalences of asthma and atopy and were exposed to a greater variety of environmental microorganisms. This diversity was inversely associated with the asthma risk (PARSIFAL: OR=0.62 [0.44-0.89], GABRIELA OR=0.86 [0.75-0.99]). Within the microbial exposure spectrum the presence of certain more circumscribed exposures were inversely related to asthma; these included exposure to the fungal taxon Eurotium sp. (aOR=0.57 [0.38-0.86]) and exposure to a variety of bacterial species including Listeria monocytogenes, Bacillus sp., Corynebacterium sp. and others (aOR=0.37 [0.18-0.76]).

Conclusion: Farm children were exposed to a wider range of microbial exposure than control children; this exposure explains a substantial fraction of the protective effect of farming on asthma. Within the broad microbial exposure spectrum several hot spots have been identified.

PROGRAM
Saturday, June 16

ADHERENT-INVASIVE *E. COLI* IN CROHN'S DISEASE

A. Darfeuille-Michaud

Université Auvergne, Clermont-Ferrand, France

The ileal mucosa of Crohn's disease (CD) patients is abnormally colonized by adherent-invasive *E. coli* (AIEC), able to adhere to and to invade intestinal epithelial cells and to replicate intracellularly. They also survive and extensively replicate within macrophages in large vacuoles and AIEC-infected macrophages secrete large amounts of TNF-α. AIEC are also able to target Peyer's patches and especially M cells via the expression of Long Polar Fimbriae. Their ability to colonize the gut of CD patients is linked to abnormal expression of carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) on the apical surface of ileal epithelial cells. AIEC adhesion is dependent on the expression of type 1 pili recognizing CEACAM6. Increased CEACAM6 expression is observed in cultured intestinal epithelial cells after infection with AIEC bacteria, indicating that AIEC are a novel example of bacterial pathogens subverting functional membrane-bound proteins as receptors for colonizing epithelia. The reference AIEC strain LF82 induces severe colitis in CEABAC10 transgenic mice expressing human CEACAM receptors and interestingly this was not observed with non-pathogenic *E. coli* K-12 or nonpiliated LF82 mutants. AIEC invasion involves the interaction of outer membrane protein OmpA with the endoplasmic reticulum stress protein Gp96, a chaperone with increased expression in CD patients. On the host side, genetic and genome-wide association studies highlighted a highly significant and replicated association between CD and variants in genes *NOD2*, *ATG16L1* and *IRGM*, all involved in the autophagy process. Functional autophagy limits intracellular AIEC replication and autophagy deficiency favors preferentially AIEC bacteria to survive and/or replicate intracellularly.

PROBIOTIC MECHANISMS

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Global patterns of the standing and active human gut microbiome in health and inflammatory bowel diseases

DETECTION, IDENTIFICATION AND QUANTIFICATION OF DIFFERENT PROBIOTIC LACTOBACILLI IN FOOD BY USING A MULTIPLEX QPCR METHOD

S.R. Herbel[1], M. von Nickisch-Rosenegk[2], M. Kuhn[3], L.H. Wieler[1], S. Günther[1]

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[2] *Fraunhofer Institute, IBMT Potsdam-Golm, Am Mühlenberg 13, 14476 Potsdam, Germany*

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Probiotic strains are often used in dairy products and in medical pharmaceuticals. They are also fed to animals, in particular since the European Union adopted a prohibition to use antibiotics to increase the immune defense of animals against bacterial infections. In general, probiotics promote beneficial influences on the gastrointestinal tract and thereby on health.

So far the different strains of the genera Lactobacillus and Bifidobacterium were isolated from different probiotic food samples and were compared with other reference strains from culture collections by phenotypic criteria and by using the polymerase chain reaction (PCR). Present methods for the isolation of probiotic bacterial are using selective media, utilizing different growth conditions – e.g. different temperatures. However, these methods are time-consuming, labor-intensive and the species differentiation via the phenotypic characterization is error-prone.

To detect and quantify more than one strain of interest in food samples, a DNA-isolation- and a molecular-based method has to be established for a species-specific characterization within an isolated DNA-mixture of probiotic strains. Therefore, the use of a usual PCR-detection method is not feasible, as this method does not enable a quantification ability of strains in the samples analysed. There clearly is a need to develop a TaqMan®-qPCR assay to detect, identify and quantify different probiotic strains by using a primer mixture.

It was shown that the formerly selected target-sequences situated within the 23s-5s rRNA (intergenic spacer) were not species-specific by using the qPCR-technique. So other sequences like the heat shock proteins (hsp60) had been chosen for the specific identification of different species of the genus Lactobacillus. The ATPase subunit of the ATP-dependent clpC-gene was selected for the species-specific identification of members of the genus Bifidobacterium.

Ongoing, we designed a TaqMan®-labelled primer pair system based on the already existing specifically working oligonucleotides for Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus helveticus, Lactobacillus johnsonii and Lactobacillus reuteri. For Bifidobacterium animalis subsp. lactis and Bifidobacterium bifidum the clpC-gene region had been tested successfully.

Currently we are testing a primer mixture for establishing a multiplex-qPCR for the Lactobacilli-species.

The advantage by using the multiplex-qPCR method is a rapid and species-specific detection, identification and quantification of different probiotic strains within one qPCR run.

TREATMENT WITH A B. BIFIDUM STRAIN AMELIORATES COLITIS IN THREE MOUSE MODELS OF COLITIS

J. Schützner[1], D. Philippe[2], J.-H. Niess[3], C.U. Riedel[1]

[1]*Institute for Microbiology and Biotechnology, Ulm University*
[2]*Nutrition and Health Dep., Nestle Research Center Lausanne*
[3]*Department of Internal Medicine I, Ulm University*

A promising application for bifidobacteria is their use as probiotic therapy in inflammatory bowel disease (IBD) since various strains reveal beneficial effects for their hosts. In previous in vitro experiments we were able to show the anti-inflammatory potential of different bifidobacterial strains using LPS-challenged intestinal epithelial cells. In the current study we compared the anti-inflammatory effects of a B. bifidum strain with good adhesive properties and a potent anti-inflammatory capacity in vitro to a non-adherent B. longum ssp. infantis strain with no anti-inflammatory capacity. Both strains were tested in the $Rag1^{-/-}$ $CD4^{+}$ T-cell transfer model, the dextran sodium sulfate (DSS) and a TNBS induced model of murine colitis. In each experiment two groups of mice (n=11-12 per group) received either B. bifidum or B. longum ssp. infantis (2×10^9 cfu/animal by oral gavage) and the colitis inducing agent. Two control groups received placebo and colitis was triggered in one of them. Feeding with bifidobacteria and placebo was continued three times a week or daily until the end of the trial when all animals were sacrificed. Mice were pre-treated with placebo or bifidobacterial strains starting one week before induction of colitis. The anti-inflammatory effect was assessed by total body weight of all animals during the trial, measurement of the colonic weight/length ratio of the dissected colons and histology scores of colonic tissue sections. Additionally, several markers of inflammation were quantified in colonic biopsies. In conclusion, treatment with the probiotic B. bifidum strain significantly alleviated the symptoms of colitis in all models whereas B. longum ssp. infantis had no effect.

ANTAGONISTIC EFFECTS OF *E. COLI* NISSLE 1917 ON VARIOUS EHEC STRAINS

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Background: In this study, the influence of the probiotic *E. coli* Nissle strain 1917 (EcN) on adhesion and growth of various pathogenic *E. coli* strains (pEc) was investigated *in vitro*. We used EAEC (O104:H4), EHEC (O157:H7), UTI (O153:H31), and two O104:H4 EHEC from the recent outbreak in Germany in our experiments.

Methods: 24-well-plates were coated with the human gut epithelial cell lines Caco2 or LS174T, to determine the adhesion of living bacteria to those cells. Single cultures of EcN, pEc, and co-cultures in ratios of 1:1 and 10:1 (EcN:pEc) were incubated for 2 h with the epithelial cells, followed by washing and plating of serial dilutions on agar plates. Furthermore, the growth of the bacteria in each well was determined at t=0h, t=2h, t=5h and t=24h by plating of serial dilutions on agar plates.

Results: We observed two effects. EcN significantly reduced the adhesion of all tested pEc strains to Caco2 and LS174T cells by up to 99 % for EAEC O104:H4, UTI O153:H31, EHEC O157:H7 and to >80 % for O104:H4 EHEC, in a co-culture with 10-fold EcN. In addition, inhibition of growth, due to killing of pathogenic *E. coli,* occurred. The number of EHEC (O157:H7) in co-culture with EcN, for example, dropped to an amount that was at least a 100-fold lower than the number of EcN after 24 h.

Outlook: EcN could be effective in preventing an infection with pathogenic *E. coli* strains (EHEC) and even assist in the treatment of acutely affected patients.

THE ROLE OF THE HOST RESPONSE IN COLICIN-DEPENDENT *E. COLI* –*SALMONELLA* COMPETITION IN THE GUT

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The host's immune system plays a key role in modulating growth of pathogens and the intestinal microbiota. Here, we investigated competition of pathogenic *Salmonella enterica* serovar Typhimurium (*S.* Tm) with commensal *E. coli* strains in the gut. Co-infection experiments using *S*. Tm and commensal *Escherichia coli* strains in a mouse colitis model showed that the pathogen can benefit from production of a bacterial toxin, colicin Ib. However, the colicin Ib dependent advantage was only observed in the presence of inflammation. Thus, we investigated how inflammation affects colicin Ib-dependent competition of *Salmonella* and *E. coli*. Both, colicin Ib and its cognate outer-membrane receptor CirA on *E. coli*, are under control of the Fur regulator and induced upon iron limitation in the inflamed gut. Increased expression of *cirA* as a response to iron depletion strongly enhanced colicin Ib-mediated killing *of E. coli.* In addition to Fur, colicin Ib expression is under control of the SOS response. Hence, under SOS stress conditions, production of the toxin is drastically increased. In *S.* Tm, iron depletion and the SOS response have a cumulative inductive effect on colicin Ib expression. Thus, inflammation fuels colicin Ib dependent competition of *S.* Tm and commensal *E. coli*. This reveals how inter-bacterial competition is modulated by the host's immune response in the gut and sheds new light on the mode of action of enterobacterial colicins.

IDENTIFICATION OF D-TRYPTOPHAN AS IMMUNOLOGICALLY ACTIVE COMPOUND EXCRETED BY PROBIOTIC BACTERIA USING IMMUNOLOGICAL *IN VITRO*-TEST SYSTEMS

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The interest of probiotic bacteria is increasing, however there is still a lack of understanding the underlying mechanisms. One reason for the detected health benefits might be the crosstalk between bacteria and the host which is difficult to predict. Therefore, we aimed to identify soluble compounds produced by probiotic bacteria using *in vitro* screening tools and high performance chemical analysis.

Gram-positive probiotic bacteria were grown in defined minimal medium and supernatants were taken at stationary phase. They were screened for their ability to prevent the lipopolysaccharide-induced maturation of human monocyte-derived dendritic cells (DC). Furthermore tests were performed indicating inhibition of the allergy-related thymus and activation regulated chemokine (TARC) secretion of KM-H2 Hodgkin Lymphoma cells. Immune-active supernatants were fractionated by solid phase extraction and elution was performed with increasing methanol concentration in water. Supernatants of several bacterial strains significantly down-regulate the expression of activation markers in DCs and the TARC secretion of KM-H2 cells. The immune-modulatory activity was found in three different methanol fractions of two selected Lactobacilli. Immune-active fractions were chemically characterized using chromatography, FTICR-MS and NMR. We focused on the 20% methanol fraction and were able to characterize D-tryptophan as a bioactive compound. In addition, it was the only D-amino acid having this immune modulatory effect even at 50nM concentration level in the test assays.

GLOBAL PATTERNS OF THE STANDING AND ACTIVE HUMAN GUT MICROBIOME IN HEALTH AND INFLAMMATORY BOWEL DISEASES

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The knowledge of species composition and functional aspects of the human microbiome is steadily increasing, but little is known regarding the variation in these factors on a global scale and how this might interact with the development of chronic diseases such as crohn disease and ulcerative colitis. General bacterial community patterns may be of clinical relevance, but the stratification of these patterns according to different human populations is of extreme importance. In this study we have analyzed a set of mucosal biopsies sampled from individuals of German, Lithuanian and Indian origin using 454 pyrosequencing of the bacterial 16S rRNA gene (DNA and cDNA). Within each geographic sample healthy controls, crohn disease and ulcerative colitis patients were included. Using ecological analyses we found strong geographic as well as global disease patterns in the abundance of major phyla and alpha diversity, which differ between the standing (DNA) and active (cDNA) bacterial community. Geography appears to dominate community clustering. However, when these geographic influences are accounted for, a universal pattern of disease status becomes apparent and reveals the importance of the interaction of geography and disease in the interpretation of disease-associated changes in microbial communities.

MANIPULATION OF THE IMMUNE SYSTEM THROUGH MICROBIOTA USING A TRANSLATIONAL ANIMAL MODEL

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The role of the complex intestinal microbiota in determining immunological function in neonates is becoming increasingly apparent. It is now well-established from studies in germ-free rodents that expansion of the mucosal and systemic immune system is limited in the absence of stimulation by microbiota. In addition, studies involving colonisation with defined strains have also demonstrated the impact of microbiota in a number of disease models in rodents, including inflammatory bowel disease and autoimmunity. These diseases occur clinically in humans and animals colonised with complex microbiotas, and in many cases variation in microbiota can be linked to the presence of disease. However, the direction of causality is often difficult to infer from epidemiological studies, and tractable, relevant animal models are needed. Characterisation of normal microbiotas in a range of species have suggested that the those of mice are very unstable, in that variation within a group of mice is low but the 'group' microbiota changes relatively rapidly over time. In contrast, gut microbiotas of pigs and humans are more variable between individuals and more stable in each individual over time. This difference may be attributable to the process of repeated re-derivation of mouse strains by caesarean section and colonisation with defined, restricted microbiotas such as the Schaedler flora.

Manipulation of the microbiota of caesarean-derived piglets can be carried out using defined colonisation with a pig-specific oligobiota, and has demonstrated direct effects on recruitment of dendritic cells initially and CD4+ T-cells secondarily. However, an advantage of the pig model is the ability to manipulate the complex microbiota of naturally-born neonates using rearing environment: since pigs have large litters and are precocial, they can be separated from the mother soon after birth. Using this model, we have demonstrated similar, early effects of rearing environment (either suckling the sow or fed milk formula in individual isolators) on microbiota and on recruitment of dendritic cells to the intestinal mucosa. In addition, these environments have significant effects on the type of CD4+ T-cells which are subsequently recruited. Isolator environments resulted in a reduction of Foxp3+ CD4+ T-cells in intestinal mucosa and an increase in antibody to novel protein fed at weaning compared to piglets housed in indoor farms, while piglets from extensive, outdoor farms were partially protected against these effects. Our current studies are focused on targeted manipulation of complex intestinal microbiota in neonatal piglets using single microbial strains or their preferred substrates.

MECHANISMS OF INFLAMMATION

14^{00} – 15^{30} Chair: C. Riedel, Institute for Microbiology and Biotechnology, Ulm University

S. Lipinski, Institute of Clinical Molecular Biology, CAU Kiel
Extracellular Cathepsin K is protective against chronic intestinal inflammation in mice and involved in host-microbe homeostasis

A. Steimle, Med. Microbiology and Hygiene, University Tübingen
Molecular mechanisms leading to semi-mature murine dendritic cells and their role in intestinal homeostasis

M. Weiher, Biofunctionality, TU Munich
Antibiotic treatment changes gut bacterial diversity and protects TNFdeltaARE mice from Crohn's disease-like ileitis

A. Schmidt, Biofunctionality, TU Munich
Dietary factors in the regulation of Crohn's disease-like ileitis

C. Manta, Dep. of Internal Medicine I + Department of Biotechnology, Ulm University
CX3CR1+ Macrophages induce the expansion of IL-22 producing CD4+RORγt+CD3- Innate Lymphocytes required for the control of a C. rodentium infection

K. Radulovic, Department of Internal Medicine I, Ulm University
The early activation marker CD69 regulates the intestinal inflammation by affecting migration of CD4 T cells and generation of Treg cells

EXTRACELLULAR CATHEPSIN K IS PROTECTIVE AGAINST CHRONIC INTESTINAL INFLAMMATION IN MICE AND INVOLVED IN HOST-MICROBE HOMEOSTASIS

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Background & Aims: Cathepsin K is a lysosomal cysteine protease known for its importance in bone resorption. Recently, a role for cathepsin K in chronic inflammatory responses became evident upon the demonstration that cathepsin K-deficient (*Ctsk*$^{-/-}$) mice are highly susceptible to experimental autoimmune arthritis and encephalomyelitis. Here, we address the relevance of cathepsin K in the intestinal immune response during chronic intestinal inflammation.

Methods: Chronic colitis was induced by three cycles of 2% DSS (dextran sodium sulfate) treatment in *Ctsk*$^{-/-}$ and WT mice. Mice were assessed for disease severity, histopathology and endoscopic appearance. Furthermore, DSS-exposed *Ctsk*$^{-/-}$ mice were subjected to rectal administration of recombinant cathepsin K. Changes in intestinal microbial signatures were assessed by using real-time quantification of fecal and colonic biopsy samples and 16S rRNA microbial fingerprinting by 454 sequencing.

Results: Here, we demonstrate a protective role of cathepsin K using a *Ctsk*$^{-/-}$ mouse colitis model. Dissecting the underlying mechanisms we found that cathepsin K is expressed by intestinal goblet cells and present in the mucin layer. Also, intestinal microbiota differed significantly between *Ctsk*$^{-/-}$ and WT mice. We found that cathepsin K exerts a direct anti-microbial activity, which potentially explains the altered intestinal microbiota observed in *Ctsk*$^{-/-}$ mice. Notably, rectal administration of recombinant cathepsin K in DSS-treated *Ctsk*$^{-/-}$ mice ameliorates the severity of intestinal inflammation.

Conclusions: Our data assign a key role to extracellular cathepsin K for maintenance of microbial colonic communities. We suggest that topical administration of cathepsin K could lead to new avenues for the development of therapies restoring intestinal homeostasis in IBD.

MOLECULAR MECHANISMS LEADING TO SEMI-MATURE MURINE DENDRITIC CELLS AND THEIR ROLE IN INTESTINAL HOMEOSTASIS

A. Steimle, J. Müller, A. Schäfer, H. Kalbacher, M. Jucker, T. Reinheckel, K. Gronbach, I.B. Autenrieth and J.-S. Frick

Institute of Medical Microbiology and Hygiene, University Tübingen

Dendritic cells (DCs) can provide different phenotypes regarding activation and maturation. They can display an immature DC (iDC) phenotype or an activated mature DC (mDC) phenotype. Recently a third phenotype has been discovered, termed semi-mature (smDCs). These smDCs are able to take up antigen, but not to process it and they show reduced expression of T cell activating co-stimulatory molecules and a reduced expression of MHC class II. SmDC fail to polarize T cells. Sm BMDCs show reduced cleaving of the invariant chain (Ii) compared to mDCs, a major regulator of the MHC class II transport to the cell surface, so leading to reduced MHC class II surface expression. The cleaving of the invariant chain is catalyzed by the endosomal protease CatS, which is regulated by the endogenous inhibitor Cystatin C. Indeed smDCs provide no regulation of CatS activity compared to iDcs whereas CatS activity of mDCs is significantly higher. This high CatS activity results in enhanced cleaving of the invariant chain and therefore efficient MHC class II transport the cell surface. Due to its unregulated CatS activity, this is not the case in smDCs.

Therefore we suggest that DC semi-maturation plays an important role in maintaining the intestinal homeostasis and that regulation of Cathepsin S could be a potential target for the treatment of colitis.

ANTIBIOTIC TREATMENT CHANGES GUT BACTERIAL DIVERSITY AND PROTECTS TNF^{DELTAARE} MICE FROM CROHN'S DISEASE-LIKE ILEITIS

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Crohn's disease is associated with dysbiosis of the gut microbiota. To test the causative role of intestinal microorganisms in the development of genetically-driven chronic inflammation and to identify bacterial taxa associated with disease, we used the TNF^{deltaARE} mouse model of Crohn´s disease-like ileitis. We wanted to show the direct effect of the ileal microbiota on ileal pathology.

Two combinations of antibiotics (Van/Met: vancomycin (0.25 g/l), metronidazole (1 g/l); Mix: Van/Met, norfloxacin (0.25 g/l), neomycin (0.25 g/l)) were administered to TNF^{deltaARE} and wild-type mice (each n = 6) in drinking water for 4 weeks. Inflammation was scored after microscopic observation of distal ileal sections. Bacterial composition was analyzed by high-throughput 16S rRNA gene sequencing.

Antibiotic treatment at early and later stages of disease development significantly inhibited tissue pathology in the distal ileum of TNF^{deltaARE} mice. In the treatment trial (starting at week 8 of age), mean scores were 5.6 ± 1.2 in control mice *vs.* 0.9 ± 0.2 in the Van/Met and 3.6 ± 0.9 in the Mix-group ($p < 0.001$). In the prevention trial (starting at week 4 of age), reduction in inflammation was not as striking (3.92 ± 0.5 *vs.* 1.85 ± 0.7 and 2.14 ± 1.0, respectively). Total bacterial counts in the ileum and the caecum were not reduced after antibiotic treatment. However, bacterial composition was markedly changed. In control mice, *Firmicutes* and *Bacteroidetes* were the dominant phyla in the caecum lumen (> 82% of total sequences). The Van/Met treatment increased the occurrence of *Proteobacteria* (26.6 ± 9.0 % *vs.* 0.8 ± 0.3), whereas the proportion of *Bacteroides* was reduced (7.2 ± 10% *vs.* 15.0 ± 6.0 %). Similar shifts in diversity were observed in ileal mucosa-associated communities, *e.g.* proportions of *Akkermansia* spp. were increased (0.1 ± 0.01% *vs.* 1.2 ± 0.02%). Bacterial diversity changes after the Mix-treatment are being currently analyzed. Metabolomic analysis of caecal content via FT-ICR/MS will allow mapping of gut metabolic networks associated with inflammation. Our findings indicate that intestinal microorganisms are essential for the development of ileitis in TNF^{deltaARE} mice. We now intend to use culture-based and gnotobiological approaches to identify specific bacterial triggers of disease.

Keywords: Intestinal microbiota, ileitis, TNF^{deltaARE} mice.

DIETARY FACTORS IN THE REGULATION OF CROHN'S DISEASE-LIKE ILEITIS

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Inflammatory bowel diseases (IBD) comprising Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing inflammatory disorders of the gastrointestinal tract. Accumulating evidence suggests that a combination of environmental factors such as smoking or diet may contribute to a dysbalanced immune-response against the commensal microflora in a genetically susceptible host. The $TNF^{\Delta ARE/WT}$ mouse resembles a model for CD, which develops chronic ileitis under conventional conditions being provided a standard Chow diet. By contrast, we could show that the pathogenesis of Crohn's disease-like ileitis could be inhibited by early dietary intervention using a semi-synthetic experimental diet (Exp). The protective state was associated with decreased expression of proinflammatory cytokines, pattern recognition receptors and homing related addressins in distal ileal tissues. Although dietary intervention was not associated with a phenotypic change of $CD8^+$ effector IEL/LPL subpopulations, we did observe an overall decrease in infiltrating leukocytes. However, administration of experimental diet was not effective for induction of remission in an already established inflammatory setting. Moreover, supplementation of experimental diet with low concentrations (10%) of Chow was sufficient to induce maximal chronic intestinal inflammation. FT-IR analysis of cecal contents from Chow and Exp treatment groups showed diet-related differences in spectral distance. However, no alteration in antigenicity could be observed in a coculture model of cecal lysate pulsed BM-DCs and $CD4^+$ T-cells. Furthermore, gluten was identified as dietary antigen that plays a role in Crohn's disease-like ileitis. Peptic tryptic digests of gluten induced TNF secretion in total MLNs and gluten fortified experimental diet could induce chronic ileitis in $TNF^{\Delta ARE/WT}$ mice. The protective effect of experimental diet inhibiting mucosal inflammation could be confirmed in the $IL\text{-}10^{-/-}$ mouse, whereas results in a T-cell transfer model of colitis seemed to be equivocal.

In conclusion, we could show that Crohn's disease-like ileitis can be inhibited by dietary intervention using a semi-synthetic experimental diet. Unraveling the underlying mechanisms might reveal new concepts for the improvement of nutritional therapy in IBD patients. Moreover, gluten fortification of experimental diet could reverse the protective effect. Thus, the $TNF^{\Delta ARE/WT}$ mouse might serve as a new model for spontaneous gluten intolerance.

CX3CR1+ MACROPHAGES INDUCE THE EXPANSION OF IL-22 PRODUCING CD4+RORγT+CD3- INNATE LYMPHOCYTES REQUIRED FOR THE CONTROL OF A C. RODENTIUM INFECTION

C. Manta, K. Radulovic, V. Rossini, C. Riedel and J.H. Niess

Department of Internal Medicine I and Department of Biotechnology Ulm University

Introduction: Innate immune cells, such as innate lymphoid cells (ILC), intestinal epithelial cells (IEC), macrophages and granulocytes provide a first line of defense to enteric pathogens. Aims and *Methods:* To study the role of CX_3CR1^+ macrophages in host defense we infected CX_3CR1-GFP animals with a red fluorescent *Citrobacter rodentium* mutant that was generated by integrating the plasmid p16S_PT5mRuby into a 16S locus of the bacterial chromosome.

Results: When CX_3CR1-GFP animals are infected with *C. rodentium* $CX_3CR1^{-/-}$ animals showed a delayed clearance of *C. rodentium* as compared to wt B6 animals as demonstrated by increased fecal counts, more sever histopathologic colitis scores, increased colon weight/colon length ratios and increased *C. rodentium* burden in liver, spleen and mesenteric lymph nodes (MLN). Red fluorescent *C. rodentium* are located within CX_3CR1^+ macrophages as shown by *ex vivo* confocal imaging. The delayed clearance of *C. rodentium* is associated with reduced numbers of IL-22 producing lymphoid-tissue inducer cells (LTi cells). The reduced IL-22 expression correlates with decreased expression of the antimicrobial peptides RegIIIβ and RegIIIγ. *C. rodentium* infection in $RAG^{-/-}$ x $CX_3CR1^{GFP/GFP}$ is associated with increased macroscopic signs of *C. rodentium*-induced colitis, increased colon weight/colon length ratios and strong reduction of IL-22 producing cells in the colonic lamina propria. The depletion of CX_3CR1^+ cells by diphtheria toxin injection in CX_3CR1-GFP x CD11c.DOG animals confirmed the role of CX_3CR1^+ macrophages in establishing IL-22 production by LTi cells.

Conclusion: The CX_3CR1^+ macrophage dependent regulation of IL-22 production may hence play an important defence mechanism to enteric pathogens such as *C. rodentium*.

THE EARLY ACTIVATION MARKER CD69 REGULATES THE INTESTINAL INFLAMMATION BY AFFECTING MIGRATION OF CD4 T CELLS AND GENERATION OF TREG CELLS

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Background: Normally about 10% of CD4 T cells in intestine are Foxp3 regulatory cells (Treg). Here, we report that the surface receptor CD69, a type II membrane glycoprotein, regulates the representation of Treg cells in intestine by affecting both their generation and the migration of CD4 T cells.

Methods: The gene expression in the intestinal tissues of B6 and CD69-deficient mice was analyzed by quantitative real time PCR (qRT-PCR).

Results: $CD69^{-/-}$ mice showed no signs of spontaneous colitis despite the reduced fraction of Treg cells and increased expression of pro-inflammatory chemokines (CCL-1, CCL-19 and CXCL-10) in intestine compared to B6 mice. Micro-array analyzes of CD4 T cells showed increased expression of chemokine genes in the absence of CD69. $CD69^{-/-}$ CD4 T cells showed *in vitro* increased chemotaxis towards CCL-1 and CXCL-10 and *in vivo* increased migration into the intestinal tissues compared to B6 cells. In Treg inducing conditions, CD69-deficiency resulted in generation of reduced numbers of Foxp3 Treg cells, both *in vivo* and *in vitro*. Administration of DSS in the drinking water induced severe colonic inflammation in $CD69^{-/-}$ mice associated with elevated levels of CCL-1, CCL-19 and CXCL-10 gene transcripts in the colon. Three days after the DSS administration $CD69^{-/-}$ mice had significantly higher numbers of CD4 T cells and lower numbers of Treg cells in the colonic lamina propria compared to B6 animals.

Conclusions: In the absence of CD69, CD4 T cells express increased levels of chemokines and migrate more efficiently to the intestinal tissues which together with difficulties in Foxp3 Treg generation result in severe colitis after the DSS treatment.

MICROBIAL CELL SURFACE GLYCOCONJUGATES AND ELICITATION OF EUKARYOTIC INNATE IMMUNITY

A. Molinaro

Organic Chemistry and Biochemistry, University Naples, Italy

Innate immunity is the first line of defence against invading microorganisms in vertebrates and the only line of defence in invertebrates and plants and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens. This mechanism relies on recognition of evolutionarily conserved structures on pathogens, termed microbe-associated molecular patterns (MAMPs), through a limited number of germ line-encoded pattern recognition receptors. MAMPs are characterized by being invariant among entire classes of pathogens, essential for the survival of the pathogen, and distinguishable from "self".

Gram negative lipopolysaccharide and peptidoglycan are two very important cell wall glyco-conjugates and act as MAMPs in eukaryotic/bacteria interactions. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Therefore, the structural study of such glyco-conjugates involved as virulence factors in animal or plant infections is a pivotal pre-requisite for the comprehension at molecular level of the innate immunity mechanisms.

In this communication I will show some examples of isolation, structure determination and elicitation and/or suppression of plant and animal innate immunity by peptidoglycan and lipopolysaccharides from pathogen and symbiotic Gram negative bacteria.

PROGRAM
Sunday,
June 17

BIOINFORMATICS ANALYSIS OF MICROBIOME SEQUENCE DATA

D. Huson

Center for Bioinformatics, University of Tübingen

Metagenomics - the study of uncultured microbes using sequencing - is a rapidly growing field in biology and medicine. Typical projects aim at understanding the role of microbes in different environments such as soil, ocean or air, and, increasingly, in the context of the human body or model organisms.

This talk will give an introduction to the field of metagenomics and will illustrate the use of some bioinformatics tools that allow one to estimate and compare the taxonomic and functional content of samples based on DNA sequences.

MECHANISMS OF INFECTIONS

10^{00} – 11^{30} Chair: B. Stecher, Max-von-Pettenkofer Institut, LMU Munich

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

M.M. Heimesaat, Inst. for Microbiology + Hygiene, Charité – University Med. Berlin
Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

A.-K.Claes, Inst. for Experimental Med., University of Kiel / Research Center Borstel
Salmonella-infection leads to exacerbated colitis in Nod2-deficient mice

E. Heupel, Institute of Microbiology and Biotechnology, University of Ulm
Citrobacter rodentium-induced colitis in mice is alleviated by pre-treatment with Bifidobacterium bifidum S17

N. Waldschmitt, Biofunctionality, TU Munich
Association of C/EBP homologous protein in intestinal epithelial cells with the development of chronic inflammation: a transgenic approach

D. Schultz, Inst. for Experimental Med., University of Kiel / Research Center Borstel
NLRP6-deficiency results in a higher susceptibility to Salmonella enterica serovar Typhimurium infection in mice

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

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

Methods: Immuno-deficient *Rag1*$^{-/-}$ mice were fed orally with EcN and transplanted with naïve CD4^{+} T cells. Weight loss and inflammatory status of the colon were monitored. Repopulation of transferred T cells in MLN and colon and DC activation were analysed by FACS. As EcN induces β-defensin production via TLR5 we also used an EcN strain deficient for the flagella (Δ*fliC*) and, in addition, used *Rag1*$^{-/-}$x*Tlr5*$^{-/-}$ mice which also were treated with EcN and transferred with T cells.

Results: EcN mediated protection from T cell transfer colitis is flagella dependent as protection was significantly reduced after gavage of flagella defective EcN (Δ*fliC*). In EcN treated *Rag1*$^{-/-}$ mice we observed a flagella dependent trapping of transferred T cells in the MLN. DC activation in cLP and MLN was similar in T cell transplanted *Rag1*$^{-/-}$ mice and *Rag1*$^{-/-}$ mice fed with EcN. Gavage of EcN only increased the amount of CD103^{+} DC in MLN. In *Rag1*$^{-/-}$*xTlr5*$^{-/-}$ mice MLN and cLP DC were less activated compared to *Rag1*$^{-/-}$ mice, suggesting a role for TLR5 signalling in DC activation. Furthermore, in *Rag1*$^{-/-}$*xTlr5*$^{-/-}$ mice EcN was no longer able to increase the amount of CD103^{+} DC suggesting an essential role for TLR5. Less activation of DC led to a significant reduced repopulation of transferred T cells which did not induce inflammation in the colon.

Conclusions: EcN flagella seems to induce CD103^{+} DC in a TLR5 dependent manner. These CD103^{+} DC seem to play a role in trapping the transferred T cells within the MLN and thereby prevent the onset of colitis.

CAMPYLOBACTER JEJUNI INFECTION OF INFANT MICE: ACUTE ENTEROCOLITIS IS FOLLOWED BY ASYMPTOMATIC INTESTINAL AND EXTRA-INTESTINAL IMMUNE RESPONSES

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Background: Campylobacter jejuni is among the leading bacterial agents causing enterocolitis worldwide. Despite the high prevalence of C. jejuni infections and its significant medical and economical consequences, intestinal pathogenesis is poorly understood. This is mainly due to the lack of appropriate animal models. In the age of 3 months, adult mice display strong colonization resistance (CR) against C. jejuni. Previous studies underlined the substantial role of the murine intestinal microbiota in maintaining CR. Due to the fact that the host-specific gut flora establishes after weaning, we investigated CR against C. jejuni in 3-weeks-old mice and studied intestinal and extra-intestinal immunopathogenesis as well as age dependent differences of the murine colon microbiota.

Methodology / Principal Findings: In infant animals infected orally immediately after weaning C. jejuni strain B2 could stably colonize the gastrointestinal tract for more than 100 days. Within six days following infection, infant mice developed acute enterocolitis as indicated by bloody diarrhea, colonic shortening and increased apoptotic cell numbers in the colon mucosa. Similar to human campylobacteriosis clinical disease manifestations were self-limited and disappeared within two weeks. Interestingly, long-term C. jejuni infection was accompanied by distinct intestinal immune and inflammatory responses as indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils, as well as apoptotic cells in the colon mucosa. Strinkingly, C. jejuni infection also induced a pronounced influx of immune cells into extra-intestinal sites such as liver, lung and kidney. Furthermore, C. jejuni susceptible weaned mice harbored a different microbiota as compared to resistant adult animals.

Conclusions / Significance: These results support the essential role of the microflora composition in CR against C. jejuni and demonstrate that infant mouse models resemble C. jejuni mediated immunopathogenesis including the characteristic self-limited enterocolitis in human campylobacteriosis. Furthermore, potential clinical and immunological sequelae of chronic C. jejuni carriers in humans can be further elucidated by investigation of long-term infected infant mice. The observed extra-intestinal disease manifestations might help to unravel the mechanisms causing complications such as reactive arthritis or Guillain-Barré-Syndrome.

SALMONELLA-INFECTION LEADS TO EXACERBATED COLITIS IN *NOD2*-DEFICIENT MICE

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The most common genetic risk factor of Crohn's disease, a chronic inflammatory bowel disease (IBD), is a mutation of the Nod-like receptor (NLR) Nod2 that recognizes the microbial structure muramyldipeptide (MDP). Here, we investigated the role of Nod2 on the development of *Salmonella*-induced colitis.

Streptomycin-pretreated C57BL/6 and Nod2$^{-/-}$ mice were orally infected with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) Δ*msbB* (altered LPS) to induce severe cecal inflammation. 2 days post infection (p.i.) inflammation was exacerbated in Nod2$^{-/-}$ mice compared to C57BL/6 mice. In particular, the edema was more pronounced and we found more neutrophils. *Salmonella* colonization remained similarly high in Nod2$^{-/-}$ mice from day 2 to day 7 p.i, whereas it significantly decreased in C57BL/6 mice. To clarify why *S.* Typhimurium Δ*msbB* induced an exacerbated inflammatory response in Nod2$^{-/-}$ mice, Nod2-, TLR2- or TLR4-transfected HEK293 cells were stimulated with heat-killed *S.* Typhimurium strains. Stimulation of TLR2-transfected cells with *S.* Typhimurium Δ*msbB* resulted in increased IL-8-production compared to *S.* Typhimurium wild type. Interestingly, IL-8-production was even more increased in Nod2-TLR2-co-transfected cells compared to single-transfected cells, indicating a synergistic effect of TLR2 on Nod2.

Infections with *S.* Typhimurium Δ*msbB* indicate that Nod2 plays an important role in the acute phase of infection controlling *Salmonella* colonization and *Salmonella*-induced inflammation. This effect is likely dependent on TLR2 signaling.

CITROBACTER RODENTIUM-INDUCED COLITIS IN MICE IS ALLEVIATED BY PRE-TREATMENT WITH *BIFIDOBACTERIUM BIFIDUM S17*

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Bifidobacteria are part of the normal human gastrointestinal flora and are widely used as probiotics in dairy and pharmaceutical products. Recent studies suggest that probiotics might be able to prevent diarrheal disorders caused by enterohemorrhagic *Escherichia coli* O157:H7 (EHEC). *Citrobacter rodentium* is an enterobacterium that causes colitis and transmissible murine colonic hyperplasia (TMCH) in mice and thus serves as a reproducible animal model of EHEC infection.

Previously, we have demonstrated that *B. bifidum* S17 has a strong anti-inflammatory capacity in different murine models of colitis ($Rag^{-/-}$ transfer, TNBS and DSS-induced colitis). In the present study, we analyzed the potential of *B. bifidum* S17 to prevent *C. rodentium*-induced colitis in mice. We observed that the body weight gain of *C. rodentium* infected mice was significantly lower compared to the uninfected control group. Pre-treatment with *B. bifidum* S17 significantly reduced the numbers of *C. rodentium* counts in faecal samples at peak of infection and later time points. The diminished body weight gain of mice infected with *C. rodentium* was ameliorated by pre-treatment with *B. bifidum* S17. Administration of *B. bifidum* S17 had minor effects on colonic lamina propria $CD4^+$ T cell subsets at the end of the experiment. In summary, this study indicates that *B. bifidum* S17 can partially ameliorate enteric infection in mice. Future experiments will demonstrate whether pre-treatment with *B. bifidum* S17 has an impact on *C. rodentium*-induced colitis especially on colonic lamina propria $CD4^+$ T cell subsets at earlier time points during *C. rodentium* infection (10-14 dpi).

ASSOCIATION OF C/EBP HOMOLOGOUS PROTEIN IN INTESTINAL EPITHELIAL CELLS WITH THE DEVELOPMENT OF CHRONIC INFLAMMATION: A TRANSGENIC APPROACH

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Background: ER stress-mediated apoptosis is linked to the expression of C/EBP homologous protein (CHOP). In this study, we characterized the role of CHOP expression in intestinal epithelial cells (IEC) in the context of inflammation.

Material and Methods: CHOP protein expression was determined in both T cell- and bacterial-mediated mouse models of colitis. Conditionally overexpressing HA-tagged CHOP (CHOP-HA) mice were generated with overexpression restricted to the intestinal epithelia. Susceptibility of $CHOP^{IEC/Tg}$ mice to DSS-induced colitis was assessed and wound healing was examined.

Results: CHOP expression levels were markly decreased in primary IEC from bacteria and T cell-mediated mouse models of colitis under inflammatory conditions. Homologous $CHOP^{IEC/Tg}$ mice did not develop signs of inflammation or tissue pathology. Stimulation with 1% DSS revealed similar susceptibility to DSS-induced colitis compared to adequate controls. $CHOP^{IEC/Tg}$ mice showed enhanced susceptibility to 2% DSS. Examination of wound healing indicates impaired function in restoration of the intestinal epithelial cell layer.

Conclusions: CHOP protein expression in the intestinal epithelium is markly reduced under conditions of inflammation. Epithelial-specific CHOP transgenic mice showed increased susceptibility to intestinal inflammation due to impaired wound healing and restoration of the intestinal epithelial surface.

NLRP6-DEFICIENCY RESULTS IN A HIGHER SUSCEPTIBILITY TO SALMONELLA ENTERICA SEROVAR TYPHIMURIUM INFECTION IN MICE

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Nucleotide-binding domain and Leucin-rich Repeat-containing Protein 6 (NLRP6) is part of the NLRP6 inflammasome. Its formation, triggered by an unknown ligand, leads to the activation of Caspase-1 which converts IL-1ß and IL-18 to their active forms. Recently, it has been shown that NLRP6 in colonic epithelial cells contributes to maintaining a healthy balance of the intestinal microbiota. Due to reduced levels of IL-18 and their altered microbiota NLRP6-deficient mice develop more severe colitis after treatment with DSS. Although inflammasomes are known to regulate intestinal inflammation during *Salmonella enterica* serovar Typhimurium infection the role of the NLRP6 inflammasome during *Salmonella* infection is still unclear.

In the present study NLRP6-deficient mice and wildtype mice were treated with streptomycin prior to infection and then infected with *Salmonella enterica* serovar Typhimurium. Pathological changes of the cecum as well as the colonization of the intestine and systemic organs were examined.

In early stages of *Salmonella*-induced colitis mesenteric lymph nodes of NLRP6-deficent mice were higher colonized with *Salmonella* compared to wildtype animals. Upon infection, both NLRP6-deficient and wildtype mice developed strong inflammation of the cecum. However, there were no significant differences in histopathology nor in intestinal colonization between wildtype and NLRP6-deficient animals. Survival curves revealed that NLRP6-deficient mice were more susceptible to *Salmonella* infection suggesting that the NLRP6 inflammasome might play a role in pathogen clearance. Future experiments will clarify how NLRP6 contributes to the control of *Salmonella*.

POSTER

1 INTESTINAL BARRIER CHANGES INDUCED BY MURINE NOROVIRUS INFECTION IN THE IL10-DEFICIENT MOUSE MODEL OF INFLAMMATORY BOWEL DISEASE

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In humans, noroviruses cause epidemic gastroenteritis. Murine noroviruses (MNV), however, will not induce disease in immunocompetent mice. We recently demonstrated that MNV infection provides a colitogenic stimulus in the IL10-deficient mouse model of IBD that depends on intestinal bacterial colonization. Aim of this study was to assess the functional and structural intestinal barrier changes induced after MNV infection in this model. *In situ* RT-PCR was used to detect presence of MNV infection in intestinal cells of C57BL/6J-*Il10*$^{-/-}$ mice. 48 hours post infection (p.i.) flux measurements were performed in miniaturized Ussing chambers and expression of barrier determining factors was assessed by qRT-PCR. Staining for apoptosis by TUNEL assay was performed 24 and 48 hours p.i. MNV was present in intestinal cells at 48 hours p.i. Mannitol flux was significantly increased in norovirus infection and expression levels of tight junction proteins were altered. Interferon inducible genes *Isg15* and *Oas1a* were significantly upregulated 48 hours after infection. Apoptosis was markedly increased in MNV infection, even more pronounced at 24 than at 48 hours p.i. MNV infection can lead to intestinal barrier changes altering paracellular permeability and expression of tight junction proteins in IL10-deficient mice. Thus, MNV infection induces epithelial barrier disruption in an IBD-susceptible host.

2 A SIGNATURE TAGGED MUTAGENESIS SCREEN TO IDENTIFY INFLAMMATION COMPETITION FACTORS OF INTESTINAL *E. COLI*

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The enteric pathogen *Salmonella typhimurium* serovar Typhimurium (*S.*Tm) is able to overgrow most of the anaerobic commensal microbiota in the gut by the induction of inflammation. Recent observations pointed out that *S.* Tm induced inflammation can induce a co-bloom of commensal *E. coli* which in turn outcompete *S.* Tm. The *E: coli* genes ("inflammation competition factors") required for *S.* Tm overgrowth in are unknown. In order to identify inflammation competition factors we will use a Signature Tagged Mutagenesis screen (STM). With this in prospect, an *E. coli* CFT073 transposon mutant library is generated and inoculated in mice in competition with *S.* Tm. In order detect attenuated mutants, we will use high-throughput sequencing of transposon insertion sites comparing the in- and output pools of the transposon mutant library. Eventually, identification of new molecular functions may open up new therapeutic approaches for the treatment of enteric Salmonella infections.

3 DEVELOPMENT OF PROBIOTIC *E. COLI* MUTANTS WITH OPTIMAL EXPRESSION OF FLUORESCENT PROTEINS FOR INTRATUMORAL IMAGING

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First successful applications of bacteria as tumor therapeutic agents were based on modified pathogens like *Clostridium perfringens* or *Salmonella typhimurium*. Unfortunately, the clinical usage of such biologics raises enormous problems with their safety and acceptance, which is why alternatives with improved biological safety profiles are required. For this purpose we investigated the applicability of probiotic intestinal bacteria as tumor therapeutic agents. Our current work focuses on the well-established *Escherichia coli* strains Nissle 1917 and Symbio G3/10. Both isolates have a proven safety record and are in use as probiotics in humans since decades. Aim of this work is to discover fundamental mechanisms of tumor colonization and destruction by using mouse tumor models and intravital microscopy. Therefore we developed several fluorescent mutants of these *E. coli* strains, which can be used to observe bacterial migration after application into the host. Because the tumor will be labelled with another fluorophore it was necessary to explore suitable dyes (blue, green, yellow, red variants and infrared) to exactly distinguish the different cell types and to visualize the interaction between bacteria and host. We can summarize that especially red variants of fluorescent proteins, like tdTomato or E2-Crimson, are suitable for *in vivo* imaging of bacteria, because they are most stable, have the greatest brightness of fluorescence and the least tendency to bleach during imaging. With regard to its long emission wavelenght, our newly established infrared sensor should be best suited for intravital microscopy, because it will enable us to investigate the probiotic bacteria in deeper tissue layers *in vivo* as compared to bacteria tagged with conventional fluorophores.

4 AN *E. COLI* STRAIN THAT COMMITS SUICIDE BY COLICIN PRODUCTION

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Colicin production is a common trait among the *Enterobacteriaceae*. Colicins are secreted into the environment under stress conditions and kill close relatives sensitive to this colicin. Thereby, they can be of advantage for the producer population under competitive situations. Commonly, the colicin producer synthesizes a protein that confers resistance to the colicin - the immunity protein. Bacteria lacking this immunity protein are not protected. We identified an *E. coli* isolate producing a “secreted factor” against which it is not protected - so the population "commits suicide" upon induction of the SOS response by mitomycin C. Using a transposon library screen as well as defined knock out mutants we found that the factor was colicin M (*cma*). We sequenced the locus for colicin M (*cma*) and colicin M immunity (*cmi*) and based on these data we hypothesize that susceptibility to colicin M is cause by a defect in Cmi. This data shed light on the mode of action of colicin M immunity as well as on evolutionary aspects of colicin production in *Enterobacteriaceae*.

5 ANTAGONISTIC ACTION OF *E. COLI* NISSLE 1917 AGAINST DIFFERENT PATHOGENIC ISOLATES FROM THE 2011 EHEC OUTBREAK IN NORTHERN GERMANY

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Background: *Escherichia coli* strain Nissle 1917 (EcN) is the active component of the pharmaceutical preparation Mutaflor®. This probiotic *E. coli* strain has been shown to inhibit the growth of certain microbial pathogens in vitro and in vivo (antagonistic action) [1]. Antagonistic effects may be due to different modes of action, e.g. the action of microcins, a competition for substrates, and the production of fitness factors, e.g. multiple siderophores. The aim of our ongoing study is to investigate whether EcN shows antagonistic activity against different subtypes of EHEC/HUSEC isolates from the latest EHEC outbreak in northern Germany in May/June 2011 and to get to know more about the modes of action of such inhibitory effects.

Methods: EcN was tested for antagonistic action against one EHEC (TY 3730) and one HUSEC strain (TY 3456) isolated from patients during the EHEC epidemic and against the EAEC strain 55989, which has the same serotype (O104:H4) as the EHEC/HUSEC isolates. In order to investigate possible antagonistic effects of EcN against these strains, we used two different assays. First, the Agar-Diffusion-Assay was used for the detection of inhibition zones produced by EcN against the different pathogenic isolates after growth on solid culture medium. Secondly, we performed competition experiments by co-culturing EcN and single pathogens in different liquid media (nutrient broth and chemically defined minimal medium) and counted the number of living bacteria at different time points to determine the degree of competition (ratio of EcN to EHEC).

Results: The Agar-Diffusion-Assays showed that EcN produced dose-dependently visible inhibition zones against all tested enterohemorrhagic *E. coli* strains. Lower concentrations of EcN bacteria led to smaller inhibition zones. Likewise, agar plates with higher amounts of the EHEC strains also showed less inhibition. Co-cultivation of EcN with the different EHEC subtypes in liquid media resulted in a shift in the ratio of the viable cell counts (EcN *vs*. EHEC) in favour of EcN during the course of the experiment (24 h). After 24 h of co-cultivation, the number of living EcN bacteria was nearly the same as in EcN monocultures, whereas the growth of EHEC strongly decreased. This antagonistic effect could be seen in nutrient broth and - even stronger – in minimal medium, and could be confirmed for both EcN:EHEC inoculation ratios (1:1 and 10:1). In both test systems, comparable antagonistic effects of EcN were found against the EAEC strain of serotype O104:H4, presumed to be the ancestor of the EHEC strains of the new outbreak.

Conclusion: Our investigation has shown that EcN exhibits antagonistic action in different assay systems against EHEC/HUSEC isolates from the 2011 outbreak in northern Germany. These findings confirm former results of others [2,3] who found inhibitory effects of the probiotic EcN strain against some other EHEC strains.

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6 IMPACT OF THE INTESTINAL MICROBIOTA ON HOMEOSTASIS

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In addition to genetic predisposition, environmental factors such as commensal bacteria contribute to the development of inflammatory bowl disease (IBD).

The gut of mammalians is colonised by a complex flora of microorganisms containing 500 – 1000 different bacterial species. These bacterial populations contribute to the health of the host, among other things, by promoting proper immune system development and limiting pathogen colonisation.

Previous studies revealed that colitis can be induced in $Rag1^{-/-}$ mice by transferring $CD4^{+}$ T-cells. This effect however seems to depend on the gut microbiota of the animals.

In the current study we therefore analyse the composition of the intestinal microbiota of $CD4^{+}$ T-cell transferred $Rag1^{-/-}$ mice by 454-Seqeuncing of 16S RNA genes.

With this method we want to reveal differences between the gut microbiota of mice that develop colitis after T-cell reconstitution compared to mice that stay healthy. Furthermore we aim to identify changes of the gut microbiota during the process of colitis development. This should give us an idea whether the gut microbiota changes because colitis develops or colitis develops due to alterations of the gut microbiota.

7 IMPROVED ADHESIVE PROPERTIES OF RECOMBINANT BIFIDOBACTERIA EXPRESSING THE *BIFIDOBACTERIUM BIFIDUM*-SPECIFIC LIPOPROTEIN BOPA

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Bifidobacteria belong to one of the predominant bacterial groups in the intestinal microflora of infants and adults. Several beneficial effects on the health status of their human host have been demonstrated, making bifidobacteria interesting candidates for probiotic applications. Adhesion of probiotics to the intestinal epithelium is discussed as a prerequisite for the persistence and the colonization of the gut.

In the present study, 15 different strains of bifidobacteria were tested for adhesion. *B. bifidum* was identified as the species showing highest adhesion to all tested intestinal epithelial cell (IEC) lines. Cell fractions of *B. bifidum* S17 were used in competitive adhesion studies with intact bacterial cells of *B. bifidum* S17 to IECs. Adhesion of *B. bifidum* S17 was strongly reduced after pre-incubation of IECs with cell wall fraction. Furthermore, after treatment of *B. bifidum* with pronase adhesion to IECs was significantly reduced. These results strongly indicate that a proteinaceous cell surface component mediates adhesion of *B. bifidum* S17 to IECs.

In silico analysis of the currently accessible *Bifidobacterium* genomes identified *bopA* as a *B. bifidum*-specific gene, encoding a lipoprotein previously identified as an adhesin of *B. bifidum* MIMBb75 (Guglielmetti *et al.*, 2008). The *in silico* results were confirmed by Southern Blot analysis. Furthermore, Northern blot analysis demonstrated that *bopA* is expressed in *B. bifidum* strains under conditions used to cultivate the strains for adhesion assays.

BopA was successfully expressed in *E. coli* BL21 (DE3) and purified by Ni-NTA affinity chromatography as a C-terminal His_6-fusion. Purified BopA had an inhibitory effect on adhesion of *B. bifidum* S17 to IECs. Moreover, BopA was successfully expressed in *B. bifidum* S17 and *B. longum/infantis* E18. Strains overexpressing BopA showed enhanced adhesion to IECs, clearly demonstrating a role of BopA in adhesion of *B. bifidum* strains.

In summary, the results of this study are one of the first reports on improved adhesive properties of *Bifidobacterium* strains.

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8 STRATEGIES FOR EFFICIENT DE-NOVO AND RE-SEQUENCING OF BACTERIAL GENOMES

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Next Generation Sequencing (NGS) technologies have revolutionised genome analysis. We have developed special libraries for sequencing with Roche and Illumina technology that allow easy assembly or mapping, scaffolding and gap closing. With long Sanger like shotgun reads produced by Roche FLX+ technology a reliable backbone of contigs for subsequent scaffolding is created. Paired end libraries with jumping distances of 3 kb, 8 kb, 20 kb and even 40 kb, sequenced with Roche FLX+ or Illumina sequencer allow to span gaps of large repetitive regions like tRNA or rRNA gene cluster. Remaining gaps can optionally be closed by PCR and Sanger sequencing. Applying advanced multiplexing of several individually bar-coded libraries allows cost efficient sequencing and assembly in parallel. In contrast to pure shotgun sequencing and mapping against a reference sequence, this strategy allows also the detection of major rearrangements, mutations and gene uptake.

9 ASSESSING *IN VIVO* COLONISATION DYNAMICS OF *B. BIFIDUM* S17 IN C57BL/6J MICE

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Bifidobacteria are inhabitants of the human gastrointestinal tract and some strains have been shown to exert a range of beneficial health effects. *B. bifidum* S17 was identified as a promising probiotic candidate strain that shows anti-inflammatory effects in several murine models of colitis (Preising *et al.,* 2010). In the presented study this strain was used to develop tools to study *in vivo* colonisation dynamics in the gastrointestinal tract of C57BL/6J mice.

For successful and selective isolation of bacteria from faecal samples, *B. bifidum* S17 was transformed with *E. coli-Bifidobacterium* shuttle vectors pMGA, pMGC, pMGE and pMGS. Based on the results of growth experiments and susceptibility of various *Bifidobacterium* strains to ampicillin, chloramphenicol, erythromycin and spectinomycin, plasmids pMGC and pMGE were selected as potential candidates for *in vivo* experiments. These plasmids were shown to replicate stable (100 %) over 100 generations in *B. bifidum* S17 but recombinant strains carrying these plasmids were recovered from faecal samples of C57BL/6J mice fed with these bacteria at very low levels. Using DGGE analysis, DNA of *B. bifidum* S17 has been detected in faecal samples collected three days post feeding. The gastrointestinal transit time of *B. bifidum* S17 pMGE was determined. This revealed that only a small amount of viable bifidobacteria can been detected within the first 24 h after inoculation of mice.

Further experiments will be required to clarify if *B. bifidum* S17 is able to persist in the intestine as viable bacteria, or if *B. bifidum* S17 does not survive the passage through the stomach and intestine and is thus not able to colonise the gut.

10 HIGH-FAT FEEDING INCREASES RECRUITMENT OF DENDRITIC CELLS TO THE LAMINA PROPRIA AND ACCELERATES DISEASE ONSET IN A MURINE MODEL OF CROHN'S DISEASE-LIKE ILEITIS

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Recent scientific findings link reduced gut barrier function to obesity-associated inflammatory co-morbidities, but there is little evidence linking high-fat diet to intestinal inflammation *per se*. The aim of this study was to elucidate the connection between diet-induced obesity, intestinal barrier functions and inflammatory processes in the intestine.
We found expression of the tight junction protein Occludin to be dramatically reduced in the ileal epithelium of BL/6 wildtype mice fed a high-fat diet (HFD) compared to control diet (CD). Consistent with increased mRNA levels of the DC chemokine CCL20 and the adhesion molecule ICAM-1 in isolated ileal epithelial cells (IEC), recruitment of $CD11c^+$ dendritic cells into the lamina propria was increased under HFD. Furthermore, HFD compared to CD accelerated ileal inflammation in $TNF^{\Delta ARE/Wt}$ mice independent of significant overweight. *In vitro* modeling revealed a higher chemotactic potential of IECs towards bone-marrow-derived dendritic cells when stimulated with cecal lysates from HFD mice compared to CD. Analysis of cecal microbial composition via pyrosequencing is under way.
These findings point out that diet rich in fat may aggravate intestinal inflammation and that this correlates to alterations of the antigen presenting cell populations present in the lamina propria and their possible interaction with intestinal epithelial cells.

11 INTESTINAL MICROBIOTA SHIFTS TOWARDS ELEVATED COMMENSAL ESCHERICHIA COLI LOADS ABROGATE COLONIZATION RESISTANCE AGAINST CAMPYLOBACTER JEJUNI IN MICE

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Background: The zoonotic pathogen Campylobacter jejuni is a leading cause of bacterial foodborne enterocolitis in humans worldwide. The understanding of immunopathology underlying human campylobacteriosis is hampered by the fact that mice display strong colonization resistance against the pathogen due to their host specific gut microbiota composition.

Methodology / Principal Findings: Since the microbiota composition changes significantly during intestinal inflammation we dissected factors contributing to colonization resistance against C. jejuni in murine ileitis, colitis and in infant mice. In contrast to healthy animals C. jejuni could stably colonize mice suffering from intestinal inflammation. Strikingly, in mice with Toxoplasma gondii-induced acute ileitis, C. jejuni disseminated to mesenteric lymphnodes, spleen, liver, kidney, and blood. In infant mice C. jejuni infection induced enterocolitis. Mice suffering from intestinal inflammation and C. jejuni susceptible infant mice displayed characteristical microbiota shifts dominated by increased numbers of commensal Escherichia coli. To further dissect the pivotal role of those distinct microbiota shifts in abrogating colonization resistance, we investigated C. jejuni infection in healthy adult mice in which the microbiota was artificially modified by feeding live commensal E. coli. Strikingly, in animals harboring supra-physiological intestinal E. coli loads, colonization resistance was significantly diminished and C. jejuni infection induced enterocolitis mimicking key features of human campylobacteriosis.

Conclusion / Significance: Murine colonization resistance against C. jejuni is abrogated by changes in the microbiota composition towards elevated E. coli loads during intestinal inflammation as well as in infant mice. Intestinal inflammation and microbiota shifts thus represent potential risk factors for C. jejuni infection. Corresponding interplays between C. jejuni and microbiota might occur in human campylobacteriosis. Murine models introduced here mimick key features of human campylobacteriosis and allow for further analysis of immunological and molecular mechanisms of C. jejuni – host interactions.

12 'TRIGGER FACTORS' SECRETED BY GRAM-POSITIVE PROBIOTIC BACTERIA EFFECT THE BARRIER FUNCTION OF INTESTINAL EPITHELIAL CELLS

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The intestinal ecosystem is characterized by a dynamic and balanced interaction between the resident microflora, the gastrointestinal barrier (GIB) and the (mucosal) immune system. In chronic inflammatory bowel disease (IBD) e.g. ulcerative colitis and Crohn's disease, this balance is deeply disturbed. Probiotic bacteria such as Lactobacilli stabilize and/or regenerate the GIB by modulating intercellular junctions and interfering with the immune response of the host.

Our recent study revealed cellular responses of intestinal epithelial cells (IECs) specifically induced by different probiotic Lactobacilli strains. Within this current project we intend to characterize signalling pathways and their target structures mediating the barrier function. In particular, we are investigating potential 'trigger-factors' that are associated with or released from the bacterial surface. We use the transepithelial electrical resistance (TER) of a T84 cell monolayer after incubation with probiotic bacteria supernatants (Lactobacilli) as a read-out system to monitor barrier integrity. We were able to show, that released components of different Lactobacilli are able to up regulate/modulate junctional complex proteins (e.g. E-cadherin and ß-catenin). Currently we are conveying our data to a mouse model (DSS colitis and RAG-/- mice).

Further insight into the underlying molecular mechanisms of the investigated ´triggerfactors´ and target molecules will foster the development of new strategies for the treatment of gastrointestinal diseases (e.g. IBD).

13 INFLUENCE OF ANTIBIOTIC TREATMENT AND MICROBIOTA ON SHIGA TOXIN-PRODUCTION BY ENTEROHEMORRHAGIC *ESCHERISCHIA COLI*

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Shiga toxin-producing *E. coli* stains are an important threat to human health as seen during the 2011 outbreak of an O104:H4 enterohemorrhagic *E. coli* (EHEC) in Germany. The Shiga toxins are encoded on lambdoid prophages and are the cause of the haemolytic uremic syndrome (HUS). In contrast to other bacterial infections antibiotic treatment of EHEC infections remains highly controversial, since antibiotics can be a trigger the activation of the lytic cycle of the prophages and can lead to an increased expression of the Shiga toxins, especially Shiga toxin 2. Different commensal gut bacteria have been shown to influence Shiga toxin either by suppression of EHEC colonization or on the other hand increasing the production by serving as a target for the released phages. We have constructed Shiga toxin 2-reporter strains of *E. coli* transduced with the Shiga toxin phage 933W. In a gnotobiotic mouse model with a low complexity gut flora, these strains will allow us to study the Shiga toxin production with a controlled and defined bacterial environment *in vivo*. We are planning to investigate the effects of antibiotic treatment, composition of commensal bacteria and host factors in our model system. The data will be helpful for the understanding of the pathogenesis of EHEC infections and the evaluation of treatment options.

14 LOSS OF UBIQUITOUS MITOCHONDRIAL CREATINE KINASE IN THE EPITHELIUM IS ASSOCIATED WITH CHRONIC INTESTINAL INFLAMMATION

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Alterations of energy homeostasis in intestinal epithelial cells (IEC) may play an important role in the pathogenesis of inflammatory bowel diseases (IBD). We recently showed that mitochondria-induced unfolded protein responses (mtUPR) contribute to the development of chronic inflammation. Transfer of high energy substrates, such as phosphocreatine, is necessary for energy shuttling out of mitochondria. In this study we investigated the role of ubiquitous mitochondrial creatine kinase (umtCK) as part of the phosphocreatine shuttle in modulating epithelial cell functions during inflammatory conditions in different animal models and human biopsies.

Proteome analysis in primary IEC from murine models of colitis identified a set of mitochondrial proteins relevant for the regulation of metabolic functions including proteins involved in ß-oxidation, respiratory chain and energy shuttling. Loss of umtCK protein expression was confirmed in primary IEC from bacteria-induced and immune-mediated models of chronic inflammation including IL-10$^{-/-}$ and Rag2$^{-/-}$ mice. In contrast, during the onset of acute inflammation in a DSS-mediated model of colitis, umtCK protein levels were increased in IEC, indicating counter regulatory mechanisms. Interestingly, during persistent DSS-challenge, sustained umtCK protein levels were associated with tissue protection, whereas loss of umtCK was associated with massive tissue damage highlighting the potential role of umtCK in maintaining tissue integrity. Remarkably, umtCK expression in the colonic murine epithelial cell line Ptk6 was cleary dependend on the cellular differentiation status, supporting the differential expression pattern of umtCK in crypt and villus epithelium of mice. In addition, the treatment of differentiated Ptk6 cells with a stabilized hydroperoxide resulted in the loss of umtCK, suggesting that the pro-oxidative environment under intestinal inflammation may affect protein stability. In conclusion, loss of umtCK as a highly susceptible protein with respect to the cellular differentiation status and oxidative stress is a consistent feature in IEC during intestinal inflammation and sustainment of umtCK correlates with protection of tissue integrity. Thus, umtCK provides a promising link between the altered IEC metabolism and the pathogensis of IBD. Future research on umtCK knockout mice will elucidate the exact role of umtCK in IEC homeostasis and its physiological role in mtUPR activation and wound healing.

15 ROLE OF TWO ESSENTIAL FACTORS OF THE PROTECTIVE EFFECT OF EcN IN DSS- INDUCED COLITIS

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The probiotic *E. coli* Nissle strain 1917 (EcN) is as effective as mesalazine in maintenance of remission in ulcerative colitis and shortens the duration of diarrhea in young children. To analyse the probiotic effects of EcN in ulcerative colitis the DSS mouse model of acute colitis was used. Therefore C57BL/6 x TLR5 littermates were fed with 3,5% DSS and either EcN, EcNΔfliC, EcNΔtcpC or EcNΔfliCΔtcpC. Every day the body weight and the disease activity index (DAI) were assessed and in the end of the experiment the colon length and the histological score were analysed. The experiments show the protective effect of EcN in the acute colitis compared to the control group. The EcN depending protection is reduced in the groups which were fed with EcNΔfliC, EcNΔtcpC or EcNΔfliCΔtcpC. So these two factors, the flagella and the protein tcpC, seem to be essential in the protective effect of EcN. To get detailed information about these two bacterial factors activation and maturation of lamina propria dendritic cells were assessed *in vivo*. Our data indicate that at least two factors, the flagella and the tcpC of EcN, account for its probiotic effects.

16 ADDITIVE EFFECTS OF EPAB AND GELATINASE ON *ENTEROCOCCUS FAECALIS* BIOFILM FORMATION AND VIRULENCE IN *C. ELEGANS* AND *G. MELLONELLA*

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Enterococcus faecalis is a commensal of the human intestinal microbiota known for harboring several putative virulence factors mediating its bacterial pathogenicity. Though being commensals, enterococci are one of the leading causes for nosocomial infections, making their virulence factors an exemplary model to analyze structure-function relationships in interaction with the host.

The impact of the enterococcal polysaccharide antigen (Epa) and the metalloprotease gelatinase (GelE) on *E. faecalis* virulence has been previously demonstrated. In this study, we characterized enterococcal virulence using a newly generated *epaB* deletion (TX5692) and a *gelE-epaB* double deletion mutant (TX5693). Both Δ*epaB* and Δ*gelE-epaB* mutants showed the same morphological phenotype with single rounded cocci. Proteolytic activity of supernatants from the *epaB* mutant was slightly decreased compared to wild type strain OG1RF. Biofilm formation under static and flow conditions was significantly diminished in the *gelE-epaB* double mutant in comparison to OG1RF and the respective single Δ*gelE* and Δ*epaB* mutant. Infection of *Caenorhabditis elegans and Galleria mellonella* with the Δ*gelE-epaB* double mutant revealed significantly attenuated mortality compared to single Δ*gelE* and Δ*epaB* mutants demonstrating potentiating effects of GelE and Epa in *E. faecalis* virulence.

17 C57BL/6J AND C57BL/6N MICE DIFFER IN THEIR GUT BACTERIAL COMMUNITY COMPOSITION

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Both C57BL/6 substrains, the C57BL/6J and C57BL/6N, are well-characterized and frequently used in metabolic research or for the generation of mouse mutants. Ongoing studies indicate that C57BL/6J males gain less weight compared to C57BL/6N when fed the same high-fat diet, despite displaying an increased food intake and energy assimilation from diet as measured by bomb calorimetry. To investigate whether these differences in dietary energy assimilation are paralleled by differences in the gut microbiota in these two closely related mouse strains, we compared their gut microbial community and carried out DNA-fingerprinting analysis on various gut segments. The results indicate that the bacterial composition differs significantly between the C57BL/6J and C57BL/6N microbiome from mice kept under SPF conditions. Furthermore, non-targeted metabolomic studies performed on mouse cecal samples using high resolution techniques such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) and Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS) detected distinct metabolic differences between both C57BL/6 substrains at the gut microbial level. Current studies involving 454 pyrosequencing analysis of the gut microbiota are expected to identify the inhabiting taxonomic groups present in the C57BL/6J and C57BL/6N gut, and therefore may provide important information about their functional roles within the complex gut network.

18 IMPACT OF DONOR-DERIVED $CD4^+CD25^+$ REGULATORY T CELLS ON GRAFT-VERSUS-HOST DISEASE OF THE GASTROINTESTINAL TRACT

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Allogeneic stem cell transplantation (SCT) is the treatment of choice for a variety of hematologic diseases. However, a significant limitation to the efficacy of allogeneic SCT is the occurrence of graft-versus-host disease (GVHD) caused by alloreactive donor T cells in the graft. The gastrointestinal (GI) tract is a major target tissue of GVHD and GI GVHD is a main cause of non-relapse mortality after SCT. To gain a better understanding of the immunological mechanisms operative in GI GVHD, intraepithelial (IEL) as well as lamina propria lymphocytes (LPL) of the small and large intestine of mice with and without GVHD after MHC-mismatched or haploidentical SCT are compared with respect to phenotype and function. Furthermore our group showed that the co-transplantation of freshly isolated donor-derived natural $CD4^+CD25^+$ regulatory T cells (Treg) prevents lethal GVHD and preliminary results indicate that Treg are also able to ameliorate ongoing GVHD. To establish mechanistic links between Treg co-transplantation and prevention/ improvement of GI GVHD, changes in the composition of the microbiota as well as within the GI-tract associated immune cell populations are investigated.

19 CX_3CR1^+ CELLS THAT HAVE PHAGOCYTOSED CFP-OVA^+*E.COLI* ARE LOCATED CLOSE TO $DSRED^+$ CD4 T CELLS

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Background: CX_3CR1^+ cells in the colon are a heterogeneous cell population consisting of $F4/80^{hi}CD11b^+$ and $F4/80^{low}CD103^-CD11b^+$ cells. Aims and Methods: To study the role of CX_3CR1^+ cells in mucosal immune responses we generated CFP-OVA^+*E.coli* (*E. coli* constitutively expressing the cyan fluorescent protein (CFP) gene linked with the gene for chicken ovalbumin protein (OVA)). After the colonization and reconstitution of CX_3CR1^{-GFP} / RAG hosts with CFP-OVA^+*E. coli* and OT-II / DsRed T cells colonic tissues were analyzed by *ex vivo* 3D confocal microscopy. Results: CX_3CR1^+ cells in the colonic lamina propria are a heterogeneous cell population that can be distinguished by F4/80 and CD11c expression. *Ex vivo* confocal 3D imaging demonstrated that CX_3CR1^+ cells from $CX_3CR1^{+/GFP}$mice and $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ extend processes into the intestinal epithelium. Fourteen days after adoptive cell transfer DsRed T cells into $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ hosts CX_3CR1^+ cells formed aggregates with DsRed CD4 T cells. After colonization of $CX_3CR1^{+/GFP}$ mice with CFP-OVA^+ *E. coli* CX_3CR1^+ cells phagocytosed CFP-OVA^+*E.coli* and activated OT-II cells as indicated IFN-γ production. CX_3CR1^+ cells (that have phagocytosed CFP-OVA^+ *E. coli)* are located close with $DsRed^+$ OT-II cells as indicated by *ex vivo* 3D confocal imaging. The activation of $DsRed^+$ OT-II cells by CX_3CR1^+ cells in $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ hosts colonized with CFP-OVA^+ *E. coli* resulted in the development of colitis as indicated by progressive body weight loss and histopathological colitis signs. Conclusion: After colon colonization of $CX_3CR1^{+/GFP}$-$RAG^{-/-}$ hosts with CFP-OVA^+*E. coli* and the reconstitution with DsRed+ OT-II T cells, mice developed colitis. The phagocytosis luminal antigens by CX_3CR1^+ cells and their interactions with CD4 T cells may regulate the integrity of the mucosal immune system.

20 ROLE OF MICRORNA IN PROBIOTIC EFFECTS OF E.COLI NISSLE 1917 : NEW PERSPECTIVES FOR TREATMENT OF INFLAMMATORY BOWEL DISEASE

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The intestinal ecosystem is characterized by dynamic interactions among its microflora, the epithelium and the immune system. An imbalance of these interactions leads to the development of inflammatory bowel diseases (IBD). The probiotic *E.coli* Nissle 1917(EcN) strain is known to have a beneficial impact on these diseases, which are aggravated, by various pathogens. However the molecular and cellular mechanisms that mediate these beneficial effects are still unknown.

We use an intestinal T84 human epithelial cell culture model to analyze cellular responses in the presence of EcN. Previous studies in our laboratory have shown that microRNAs are involved in maintaining intercellular junctions and barrier integrity of the epithelium. Three such microRNAs were proven to be affecting regulatory as well as structural proteins of the junctional complex (ZO-2, PAR-3, PAR-6), contributing towards enhancement of the epithelial barrier integrity, which is demonstrated by an increase of the transepithelial resistance of T84 monolayers.

Based on miRNA array data and future next generation sequencing (NSG) of transcription profiles of intestinal epithelial cells we wish to identify novel microRNAs that are differentially regulated under EcN incubation or EPEC infection. The miRNA expression profiles and their putative targets are validated by real time PCR and overexpression/knockdown functional studies. This will shed light on their role in maintaining barrier integrity and facilitating host immune responses. In addition, we will validate our data in several *in vivo* mouse models for IBD (DSS, $RAG^{-/-}$ mice). Detailed insight into the molecular targets of specific microRNAs might foster the development of new strategies for the treatment of gastrointestinal diseases.

21 EPITHELIAL LAYER DAMAGE MODULATES THE RECOGNITION OF BACTERIAL PRODUCTS BY RESIDENT HUMAN INTESTINAL LAMINA PROPRIA CELLS

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Human resident intestinal lamina propria cells express only low levels of pattern recognition receptors (PRR). Their mode of activation in the case of intestinal infections therefore remains obscure. Here, we investigated the interrelationship between epithelial layer damage, bacterial stimuli and inflammatory response of colonic mucosal cells using an organ culture model.

Punches of intact human intestinal mucosa or injured mucosa denuded of epithelial cells were cultured in the presence or absence of E. coli lysates. After 12h, cytokine and surface receptor expression were determined in mucosal tissue as well as in leukocytes emigrated from the injured tissue.

Intact mucosa constitutively released only low amounts of IL-1β, TNF-α, IFN-γ, and IL-22 into the organ culture supernatant. Exposure to E. coli lysates did not significantly affect production of these cytokines. In contrast, injured mucosa denuded of epithelial cells released detectable levels of IL-22, which were further increased in the presence of bacterial lysates. Secretion of IL-1β, TNF-α, and IFN-γ by the injured mucosa was low to undetectable in the absence but clearly induced in the presence of E. coli lysates. Mucosal expression of CD14, TLR2, and CD86 was up-regulated following epithelial layer damage. It was also increased in mononuclear cells emigrated from the damaged tissue when compared to resting mononuclear mucosal cells. Exposure to E. coli lysates did not further enhance expression of these receptors.

In conclusion, epithelial layer damage modulates the reactivity of resident human lamina propria cells towards bacterial stimuli. It may promote recognition of bacterial products by up-regulating PRR in these cells.

22 EXPRESSION OF THE BLOOD-GROUP-RELATED GLYCOSYLTRANSFERASE *B4GALNT2* INFLUENCES THE INTESTINAL MICROBIOTA AND SUSCEPTIBILITY TO *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM IN MICE

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Glycans on mucosal surfaces play an important role in host-microbe interactions. Bacteria do not only use host glycans as carbohydrate source, but also as attachment sites. The *B4galnt2* gene encodes a blood-group-related glycosyltransferase that is subject to strong selective forces in natural house mouse populations whereby a common allelic variant exists that results in loss of *B4galnt2* gene expression in the gastrointestinal (GI) tract. With respect to *B4galnt2* genotype, significant changes in composition of the intestinal microbiota have been identified indicating a previously unappreciated role for *B4galnt2* in host-microbial homeostasis in the gut. In addition, numerous *B4galnt2*-dependent differences in the abundance of specific bacterial taxa have been detected, including species belonging to the genus *Helicobacter*, suggesting bacterial interaction with B4galnt2 glycans. To determine whether *B4galnt2* expression also influences host susceptibility to enteric pathogens, we have applied a model of systemic infection with *Salmonella enterica* serovar Typhimurium. Based on the intestinal colonization and cecal histopathology, we found a significant influence of *B4galnt2* genotype during the early phase of infection. Furthermore, infection was associated with dynamic changes in the intestinal tissue-specific expression pattern of *B4galnt2*. These results support the hypothesis that variation in *B4galnt2* GI expression may alter susceptibility to diseases such as infectious gastroenteritis.

23 TWO WEEKS' USE OF THE SALINE LAXATIVE MAGNESIUM SULFATE CAUSES MAJOR CHANGES IN INTESTINAL MICROBIOTA

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Saline purgatives have been proposed as health remedies for centuries. Magnesium sulfate as a purgative is part of the F. X. Mayr cure popular in central European countries. The idea advanced by psychiatrists 100 years ago was that cleansing of the intestine could prevent "autointoxication." We investigated the influence of magnesium sulfate (Epsom salt) on the composition of the intestinal microbiota in 4 healthy subjects (2 females, 2 males, mean age 45 years, range 36-60). For 2 weeks, the volunteers took $MgSO_4$ to cause watery to mushy stools. Stool samples were obtained at baseline, upon termination of $MgSO_4$, and 1 month thereafter.

The intestinal microbiota was analyzed by microbial community profiling targeting the variable V1/2 region of the 16S rRNA gene and barcoded pyrosequencing with an automated system (Roche FLX /454). Analysis at baseline revealed two main patterns with a predominance of Firmicutes in 2 subjects and considerably increased Bacteroidetes counts in the other two. Regardless of the initial bacterial composition, saline purgatives caused marked changes with either a decrease of about 30% or an increase of about 26% in the Bacteroidetes. Correspondingly, Firmicutes increased or decreased to fill the gap since other phyla did not change appreciably. One month after discontinuation of the laxative, a partial reversal to the original bacterial community pattern could be identified in two participants. Furthermore, we observed a tendency in all 4 subjects to reach a similar distribution pattern with low levels of Bacteroidetes and an increase in Firmicutes.

We conclude that a 2 weeks' use of a saline laxative causes major changes in the composition of the intestinal microbiota with some persistent changes one month after discontinuation of purgatives. Additionally, the induced diarrhea appears to tend to equalize the different initial microbiota.

24 EXPRESSION ANALYSIS OF THE PILI-ENCODING GENES OF BIFIDOBACTERIUM BIFIDUM S17

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Beneficial effects of probiotic bacteria are to some extent determined by their ability to successfully colonize the gastrointestinal tract, tightly adhere to the intestinal epithelial cells and successful compete with other microorganisms present in this habitat. Specific structures on the bacterial cell surface, known as pili, were shown to play an important role in host colonization of pathogenic bacteria. Recently, gene clusters encoding for pili were identified in the genomes of different Bifidobacterium species and were shown to be involved in colonization of mice by a B. breve strain.

Here, we show that B. bifidum S17, a promising strain, which is able to tightly adhere to intestinal epithelial cells in vitro and shows anti-inflammatory activity in several murine models of colitis, possesses three pili-encoding gene clusters. Expression of the genes of these pili cluster was probed by RT-PCR in bacteria grown in vitro in MRS medium supplemented with either glucose, fructooligosaccharides, human milk serum or bile salts. Our results indicate that the genes of cluster III (BBIF_1760-1762) and cluster II (BBIF_1648-1650) are constitutively expressed under all conditions tested. By contrast, two genes in cluster I (BBIF_301 and BBIF 303) were not expressed under the conditions examined. Moreover, RT-PCR targeting the intergenic regions between all genes in these clusters confirms the transcription of clusters III as operon.

Collectively, the results indicate presence of pili structures on the surface of B. bifidum S17, which in the future will be further investigated by atomic force microscopy.

25 TOLL-LIKE RECEPTOR 2 AND 4 SIGNALING AMELIORATES DSS COLITIS BY INDUCTION OF CD103 EXPRESSING DENDRITIC CELLS

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Tolerance towards the intestinal microbiota is impaired in IBD patients, which leads among other factors to a strong and long lasting inflammation irregularly interrupted by short remission phases. In order to investigate the role of dendritic cells and their TLR2 and TLR4 expression while acute phase inflammation the dextran sulfate sodium (DSS) model was employed. Therefore either C57BL/6 mice were DSS only administered or DSS administered and bacteria fed for 6 days. Incorporated strains were *E. coli* JM83 wild type, *E. coli* JM83 Δ*htrB* $htrB_{Pg}$ mutant, featuring an altered Lipid A structure resulting in a weaker TLR4 signaling, *L. plantarum* WCSF1 wild type, as well as *L. plantarum* Δ*dltX-D* mutant, lacking D-alanylation at its lipoteichoic acid leading to a weaker TLR2 response. Feeding of wild type bacteria, capable of a potent TLR signaling induction, during DSS administration was able to prevent of intestinal inflammation by the means of weight loss, disease activity index and colons shrinking, whereas feeding of mutant bacteria, being weak inducer of TLR response, was not able to prevent of disease. TLR2 and TLR4 signaling mediated protection due to bacteria feeding was independent of activation and maturation levels of lamina propria dendritic cells (LPDC). However, significantly increased levels of CD103 positive DC are associated with prevention of inflammation. Therefore we conclude that during DSS colitis TLR2 and TLR4 signaling results in an increased level of $CD103^+$ DC and these being able to prevent of intestinal inflammation.

26 ANALYSIS OF PILUS-ENCODING GENE CLUSTERS IN TWO BIFIDOBACTERIAL SPECIES

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Tight adhesion to the intestinal epithelial cells (IECs) is considered to be an important prerequisite for host-colonisation by bacteria. For this presence of specific structures on the cell surface, pili, was shown to be important. Here we have investigated expression and organization of gene clusters encoding for pili under *in vitro* conditions in *Bifidobacterium bifidum* S17 and *B. breve* S27. *B. bifidum* S17, a promising probiotic candidate, is able to tightly adhere to the IEC and has a potent anti-inflammatory *in vitro* and *in vivo*. By contrast *B. breve* S27 does not adhere to IECs.

Cell surface of both strains was investigated by the transmission and scanning electron microscopy. Also the genomes of *B. bifidum* S17 and *B. breve* S27 were sequenced, assembled and manually annotated. Genome sequencing revealed differences in amount and structure of the potential pili-encoding clusters between *B. bifidum* S17 and *B. breve* S27 under *in vitro* conditions. *B. bifidum* S17 harbors four potential pili-encoding gene clusters: three *fim* clusters and one *tad* cluster. All genes of the *fim2* and *fim3* clusters are expressed in bacteria under conditions of standard cultivation indicating that functional pili might be expressed on the surface of *B. bifidum* S17. Analysis of the *B. breve* S27 genome revealed the presence of two *fim* clusters and one *tad* cluster. Our results indicate that the genes of only one cluster are expressed, whereas the gene encoding the minor pilin protein might be non-functional due to a frame-shift mutation. No transcripts were detected for the gene encoding the pre-pilin precursor of the Tad pili in both strains ruling out their presence on the surface under *in vitro* conditions.

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