1.

Tagung der DGHM Fachgruppe

Mikrobiota, Probiotika und Wirt
Microbiota, Probiotics and Host

2.- 4. Mai 2008
Kultur und Bildungszentrum
Kloster Seeon / Chiemsee

Fotostudio Christoph Vohler, München

Kick-off Meeting

„Mikrobiota, Probiotika und Wirt"

Vom 2.-4. Mai 2008 fand im Kultur- und Bildungungszentrum Kloster Seeon die Auftaktveranstaltung der neugegründeten Fachgruppe „Mikrobiota, Probiotika und Wirt" der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) statt. Diese neue Fachgruppe der DGHM stellt ein interdisziplinäres Forum dar, das sich mit der Rolle von nicht-pathogenen oder kommensalen Mikroorganismen und deren Interaktionen mit dem Wirt bei der Induktion oder Prävention chronisch entzündlicher, atopischer oder metabolischer Erkrankungen beschäftigt.

Mit der Gründung der neuen Fachgruppe, ist es Herrn Prof. Dr. D. Haller, der im Frühjahr die Leitung des Lehrstuhls „Biofunktionalität der Lebensmittel" an der TU München übernommen hat, gelungen, Arbeitsgruppen aus den unterschiedlichen Fachdisziplinen Mikrobiologie, Ernährungswissenschaft, Immunologie und Medizin zusammenzuführen, und dem Thema Mikroben und Wirt eine neuen Plattform zu bieten. Der neu gewählte Vorstand setzt sich aus Frau Dr. J. Frick (Universität Tübingen) und Herrn Prof. Haller zusammen, Schriftführer ist Herr Dr. Ch. U. Riedel (Institut für Mikrobiologie und Biotechnologie, Universität Ulm).

Eröffnet wurde die Veranstaltung von Tore Midtvedt (Karolinska Institute, Stockholm, Schweden), einem der ganz frühen Pioniere der **Gnotobiologie**. Ger Rijkers (University Medical Center Utrecht, Netherlands) und Brent Polk (Vanderbilt, Nashville, USA) führten die insgesamt 65 Teilnehmer der Konferenz in die **klinischen und molekularen Wirkmechanismen probiotischer Mikroorganismen** ein. Drei weitere „State-of-the-Art" Vorträge gaben Einblicke in die Welt der **regulatorischen T-Zellen** (Manfred Kopf, ETH Zürich, Switzerland), **Signaltransduktion im Darm** (Jerry Wells, Wageningen University and TNO, Netherlands) und der Wirkung von **Schwefelwassestoff als Neuromodulator** (Michael Schemann, TU Munich, Germany).

Zusammen mit den wissenschaftlich hochrangigen Beiträgen der teilnehmenden Arbeitsgruppen war es ein spannender Auftakt für die neue Fachgruppe.

Für weitere Informationen wenden Sie sich bitte unter DGHM@wzw.tum.de an Prof. Haller, oder besuchen Sie die DGHM-Homepage http://www.dghm.org/red/fachgruppen/.

Prof. B. Polk (front) listening to the short presentations in English

Prof. M. Schemann giving a talk about the neuromodulator hydrogen sulfide

PROGRAM Friday, May 2

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

17^{15} - 18^{15}	Keynote Lecture: **T. Midtvedt**, Laboratory of Medical Microbial Ecology, Karolinska Institute, Stockholm, Sweden ***Gnotobiology - A way to go for predicting the future?***

18^{15} - 19^{00}	DGHM Elections
19^{00} - 20^{45}	Dinner

MICROBIOTA AND IMMUNE FUNCTION

20^{45} - 22^{15} Chair: I. Autenrieth, Inst. of Med. Microbiology & Hygiene, Tübingen

J. Frick, Institute of Med. Microbiology & Hygiene, Tübingen
DC maturation and IL-6 production govern tolerance induction to commensal bacteria

A. Diefenbach, Institute of Med. Microbiology & Hygiene, Freiburg
Role of the commensal microflora in the education of a functional and self-tolerant natural killer cell repertoire

S. Cording, Experimental Rheumatology/Immunoregulation, Charité, Berlin
Stay with friends: commensal microflora drives expansion of FOXP3+ regulatory T cells in gut-associated lymphoid tissue

J. Buer, Med. Microbiology, Essen
Regulation of pathogen-specific immune responses at mucosal interfaces

U.B. Göbel, Inst. of Microbiology & Hygiene, Charité, Berlin
Role of commensal gut bacteria and innate immunity in gastrointestinal inflammation

A. Batra, Medizinische Klinik I, Charité, Berlin
Bacterial translocation and response of mesenteric preadipocytes to bacterial antigens

PROGRAM Saturday, May 3

08^{30} - 09^{30} Keynote Lecture: **D.B. Polk**, Department of Pediatrics, Division of Gastroenterology and Nutrition, Nashville, USA
Probiotic-derived soluble proteins prevent intestinal epithelial cell apoptosis

09^{30} - 10^{00} Coffee Break

PROBIOTIC SECTION

10^{00} - 11^{45} Chair: U.B. Göbel, Inst. of Microbiology & Hygiene, Charité, Berlin

I. Kübler, Innere Medizin, Robert-Bosch-Krankenhaus, Stuttgart
Probiotic E. coli (Symbioflor®2) treatment mediates antimicrobial ß-defensin (hBD-2) synthesis and fecal excretion in humans

S. Seifert, Dep. of Physiol. & Biochem. of Nutrition, Max Rubner-Inst., Karlsruhe
The effect of consumption of fermented milk containing Lactobacillus casei Shirota on innate immune functions in healthy men with a low natural killer cell activity

M. Lawrenz, Dep. of Immunology, MPI for Infection Biology, Berlin
A proteasome inhibitor secreted by probiotic bacteria blocks inflammatory signaling

L. Schulze, Division of Medicine, Ardeypharm GmbH, Herdecke
Successful therapy of unspecific prolonged diarrhoea in infants and toddlers with the probiotic E.coli Nissle 1917

U. Sonnenborn, Division of Biological Research, Ardeypharm GmbH, Herdecke
Investigation of the antimutagenic activity of probiotic E.coli Nissle 1917 against 4-nitroquinoline-1-oxide

S. Ehnert, Clinic for Traumatology, MRI, TU Munich
Effects of probiotics and/or immuno-nutrition on the immune system and the one-year survival of elderly patients with proximal femur fractures

C. Reiff, Gut Immunology, The Rowett Research Institute, Aberdeen, UK
VSL#3 attenuates inflammatory gene expression in the cecum of IL10KO mice

PROGRAM Saturday, May 3

12^{00} - 14^{30} Lunch

14^{30} - 15^{00} Keynote Lecture: **G.T. Rijkers**, Dep. of Surgery, University Medical Center Utrecht, Netherlands
Probiotics in acute pancreatitis: design and outcome of a multicenter trial

EPITHELIAL CELL BIOLOGY

15^{00} - 16^{30} Chair: J. Buer, Inst. für Med. Mikrobiologie, UK Essen

A. Wullaert, Dep. of Mouse Genetics & Inflam., University of Cologne
Role of NF-κB activation in intestinal epithelial cells in intestinal immune homeostasis

K. Mair, Klinikum Rechts der Isar, Gastrolabor, TU Munich
The role of intestinal epithelia for the innate immune response to non-invasive facultative pathogens

C. Cichon, Dep. of Infectiology, ZMBE, University of Münster
DNA array analysis of T84 epithelial cells co-incubated with gram-negative or gram-positiv probiotic bacteria: new perspectives for inflammatory bowel disease treatment

N. Steck, Biofunctionality, TU Munich
Enerococcus faecalis metalloprotease contributes to the development of chronic intestinal inflammation through impairment of epithelial barrier function

G. Hörmannsperger, Biofunctionality, TU Munich
Post-translational inhibition of IP-10 protein secretion in intestinal epithelial cells through ubiquitin-mediated mechanisms: bacterial strain-specific effects of VSL#3

D. Ghadimi, Inst. for Physiol. & Biochem. of Nutrition, BAfM, Kiel
The anti-inflammatory effects of probiotic DNA seem to be mediated by TLR9 in enterocytes

PROGRAM Saturday, May 3

16^{30} - 17^{00} Coffee Break

17^{00} - 17^{40} Keynote Lecture: **M. Kopf**, Inst. for Integrative Biology, ETH Zürich
CD4 T cell subsets and responses including Tregs and Th17

BACTERIAL FUNCTION

17^{40} - 19^{10} Chair: M. Blaut, Gastrointestinal Microbiology, DIFE, Nuthetal

E. Domann, Inst. of Medical Microbiology, Giessen
Comparative genomic analysis for the presence of potential enterococcal virulence factors of the probiotic Enterococcus faecalis Symbioflor 1

M. Blaut, Gastrointestinal Microbiology, DIFE, Nuthetal
Simplified models for mechanistic studies on host-microbe interactions

S. Wohlgemuth, Gastrointestinal Microbiology, DIFE, Nuthetal
Role of gut bacteria in intestinal inflammation of the IL-10$^{-/-}$ mouse

A. Schwiertz, Institute of Microecology, Herborn
Microbiota, SCFA in lean and overweighed healthy subjects

C.U. Riedel, Institute for Microbiology & Biotechnology, University Ulm
argD-dependent quorum sensing affects biofilm formation, virulence and global gene expression profiles in Listeria monocytogenes

H.-D. Grimmecke, Laves-Arzneimittel, Schötz, Switzerland
Growth physiology and biosynthesis of biogenic amines and γ-Aminobutyric acid by human, commensal / probiotic strains of Escherichia coli

19^{30} Dinner

PROGRAM Sunday, May 4

08^{30} - 09^{30} Keynote Lecture: **J. Wells**, University Wageningen
Innate signaling by intestinal microbes and its relevance to epithelial integrity

09^{30} - 10^{00} Coffee Break

CHRONIC INTESTINAL INFLAMMATION

10^{00} - 11^{00} Chair: D. Haller, Biofunctionality, TU Munich

M. Koslowski, Dr. Margarete Fischer-Bosch Inst. of Clin. Pharmacol., Stuttgart
Selective influence of Tcf-4 mediated WNT signaling on intestinal innate and adaptive immunity of ileal Crohn´s disease

M. Renner, Division of Molecular Genome Analysis, DKFZ, Heidelberg
DMBT1 confers mucosal protection in vivo and a deletion variant is associated with Crohn´s disease

K. Menzel, Department of Internal Medicine I, University of Regensburg
Paraepithelial passage of adherent luminal bacteria via modified cell-cell-contacts in Crohn´s disease

A. Messlik, Biofunctionality, TU Munich
Interleukin 10 inhibits endoplasmatic reticulum and mitochondrial unfolded protein stress responses in the intestinal epithelium: impact on chronic inflammation

11^{00} - 11^{30} Keynote Lecture: **M. Schemann**, Humanbiologie, TU Munich
Hydrogen sulfide as a novel neuromodulator: Implications for functional gut disorders

11^{30} Lunch

GNOTOBIOLOGY – A WAY TO GO FOR PREDICTING THE FUTURE?

Tore Midtvedt

Department of Microbiology, Tumor and Cell biology (MTC) Karolinska Institutet, Stockholm, Sweden

In the mid 1890ties, the two German scientists, Nuttal and Thierfelder, demonstrated that germfree guinea pigs could live – for a short period of time - without any bacteria, thereby contradicting the famous statement made 10 year earlier by L Pasteur that “life is not possible without bacteria”. However, it took 50 more years untill the first 2nd generation of germfree animals was born (August 1945, at Notre Dame University, USA). The 1960ties were glorious days for germfree animal research. Man was entering the space, and germfree technology was needed for protection from dangerous microbes in outer space.

After some years of less public attention, but hard work in made germfree laboratories around the world, the field is entering the public stage again. The possibilities of making specifically designed research animals, (transgenes and knockouts) have giving us new ways of studying the many “cross-talks that exists between the host and its gastro-intestinal microflora. This approach has between of great value in research related to inflammatory bowel diseases.

At present, it seems to be a world-wide increase in disorders as atopic eczema, adiposity and autisms in childhood. In all these three – widely different areas - alterations in development and functions of a normal intestinal microflora have been put in focus for etiopathogenetic considerations, and works on gnotobiotic animals (animals with a known flora) have been initiated Basic principles as “windows” for establishment, succession in establishment and long-term effect of establishment of some members of our normal microflora will be given.

DC MATURATION AND IL-6 PRODUCTION GOVERN TOLERANCE INDUCTION TO COMMENSAL BACTERIA

Julia-Stefanie Frick (MD)

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

Available Modelsystems:
Transgenic mice: OT-I, OT-II, DO11.10
K.O. mice: TLR (2,4), MyD88, Trif, IFN□, IL-2, IL-6, TNF, Rag
Microorganisms: *E. coli* mpk, *B. vulgatus* mpk, *Y. enterocolitica*, *B. adolescentis*, *L. fermentum*

Our main interest is the modulation of intestinal inflammatory and infectious diseases by commensal bacteria and the role of dendritic cells in immunomodulatory processes.
As IL-2-deficient (IL-$2^{-/-}$) mice mono-colonized with *E. coli* develop colitis but IL-$2^{-/-}$ mice mono-colonized with *B. vulgatus* do not, we investigated if stimualtion with *E. coli* or *B. vulgatus* differentially modulates distribution, activation and maturation of dendritic cells (DC) in vitro and in vivo. Stimulation of DC with *E. coli* induced TNF-□, IL-12 and IL-6 secretion and expression of activation-markers, increased T-cell activation and led to Th1 polarization. Stimulation with *B. vulgatus* only led to secretion of IL-6 and DC were driven to a semi-mature state with low expression of activation-markers, did not promote Th1 responses and *B. vulgatus*-induced semi-mature DC were nonresponsive to stimulation with *E. coli.* This effect was abrogated by addition of anti-IL-6-mAb or mimicked with rIL-6. This data suggests that *B. vulgatus*-induced IL-6 drives DC to a semi-mature state, thus nonresponsive to proinflammatory activation by *E. coli*. In vivo, prior to onset of colitis we found increased expression of the activation marker CD40 on lamina propria (LP) DC from *E. coli* but not *B. vulgatus* mono-colonized mice but high MHC class II expression by DC from both groups. Chemokine receptor (CCR) 7 surface expression was more strikingly enhanced in mesenteric lymph node (MLN) DC from *E. coli* than *B. vulgatus* mono-colonized mice. *B. vulgatus*-triggered IL-6 secretion by LP DC thus induces a semi-mature phenotype of DC characterized by low expression of CD40 and CCR7 but high expression of MHC class II that prevents colitogenic T cell activation. High IL-6 mRNA expression

was evident in *B. vulgatus* mono-colonized IL-2$^{-/-}$ mice that did not develop colitis. The data provide new evidence that IL-6 can act as an anti-inflammatory cytokine in the mucosa by targeting local DC.

An increasing body of evidence suggests that probiotic bacteria are effective in the treatment of enteric infections although the molecular basis of this activity remains elusive. We tested commensal *B. adolescentis* in terms of toxicity, invasiveness, inhibition of *Yersinia* induced inflammation *in vitro* and *in vivo* and modulation of dextran-sodium-sulfate (DSS) induced colitis in mice. *B. adolescentis* was neither toxic for nor invaded into epithelial cells (EC). Interestingly *B. adolescentis* inhibited *Y. enterocolitica* induced NF-□B activation and IL-8 production in EC. In line with these findings *B. adolescentis* fed mice had significantly lower mean pathogen burden in visceral organs and loss of bodyweight upon oral infection with *Y. enterocolitica*. The expression of enterocytic TNF-□ and CxCl1 was significantly diminished in mice treated with *B. adolescentis*. In addition, administration of *B. adolescentis* decelerated inflammation upon DSS treatment in mice.

We suggest, that commesal bacteria might play an important role in the modulation of infectious and inflammatory processes in the intestine.

ROLE OF THE COMMENSAL MICROFLORA IN THE EDUCATION OF A FUNCTIONAL AND SELF-TOLERANT NATURAL KILLER CELL REPERTOIRE

Stephanie Sanos[1], Caroline Johner[2], and Andreas Diefenbach[1]

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Scientific Interests:
Innate Immunity, Innate Immune Receptors, Natural Killer Cells, Dendritic Cells, Mucosal Immunity

Natural killer (NK) cells are important effector cells of the innate immune system that play a vital role in the protection against viruses and tumors. NK cells were long viewed as “naturally active” innate immune cells that follow a cell-autonomous activation program and do not require interaction with others cells for gaining full functionality. However, recent evidence by us and others indicate that NK cells would be better described as rapidly acting lymphocytes that need priming for proper activation and undergo an education program that has been compared to positive selection of T cells in the thymus. During this education program, NK cells are probed for the acquisition of inhibitory receptors reactive with “self”-class I MHC molecules. NK cells acquiring such receptors become functional, those who fail during this education process become anergic. Interestingly, NK cells of newborn mice are not functional and do not express any inhibitory receptors which they acquire during the first 6-8 weeks after birth. The signals inducing the upregulation of inhibitory receptors and, thus, the education of functional NK cells are unknown. We have probed the role of the commensal microflora in this process by comparing NK cell education and function in germfree and specific pathogen-free mice. I will report on the results of these studies and their implications for our understanding of how NK cells develop a functional but yet self-tolerant population of effector cells.

STAY WITH FRIENDS: COMMENSAL MICROFLORA DRIVES EXPANSION OF FOXP3$^+$ REGULATORY T CELLS IN GUT-ASSOCIATED LYPHOID TISSUE

Siewert, Christiane[1]; Cording, Sascha[1]; Heimesaat, Markus M.[2]; Liesenfeld, Oliver[2]; Bereswill, Stefan[2]; Loddenkemper, Christoph[3]; Loh, Gunnar[4]; Blaut, Michael[4]; Hamann, Alf[1] and Huehn, Jochen[1]

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Scientific Interests and available Modelsystems:
immunoregulation, regulatory T cells (Tregs), commensal microflora, TLR signaling
germ-free mice, gnotobiotic mice, several knock-out mice (TLR2$^{-/-}$, TLR4$^{-/-}$, TLR2/4$^{-/-}$, TLR9$^{-/-}$)

Colitis models have provided compelling evidence for a protective role of Foxp3$^+$CD25$^+$CD4$^+$ regulatory T cells (Tregs) in intestinal homeostasis. Foxp3$^+$ Tregs have been described as thymus-derived cells, however, more recent studies demonstrate a significant peripheral turnover.
Here we investigated the proliferation of Foxp3$^+$ Tregs in gut-associated lymphoid tissues (GALT) and analyzed the contribution of the commensal microflora. Thereto, mice were treated with a cocktail of antibiotics to deplete the commensal microflora. This treatment led to a significant reduction of CD4$^+$Foxp3$^+$ Treg numbers not only in the GALT, but also in spleen and peripheral lymph nodes. Analysis of the *in vivo* proliferation of Foxp3$^+$ Tregs by BrdU-incorporation revealed a significantly reduced frequency of cycling BrdU$^+$Foxp3$^+$ Tregs solely in the GALT, but not in spleen and peripheral lymph nodes. These findings indicate that the commensal microflora contributes to the local proliferation of Foxp3$^+$ Tregs, which influences the Treg numbers systemically. Analysis of frequencies, absolute cell numbers and homeostatic proliferation of Foxp3$^+$ Tregs in TLR2$^{-/-}$, TLR4$^{-/-}$, TLR2/4$^{-/-}$ and TLR9$^{-/-}$ mice showed that sensing of bacterial stimuli via these TLR's is not the major mechanism controlling intestinal homeostasis of Foxp3$^+$ Tregs.
Together, our data show that microbial stimuli critically influence the homeostasis of Foxp3$^+$ Tregs and suggest that these cells, which protect against intestinal inflammation, might not exclusively consist of self-reactive T cells.

REGULATION OF PATHOGEN-SPECIFIC IMMUNE RESPONSES AT MUCOSAL INTERFACES

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Scientific Interests:
Immune regulation, Mucosal Immunity, Tregs,

The intestinal immune system has evolved redundant regulatory mechanisms to prevent unwarranted inflammation due to host-pathogen interactions. This involves regulatory T cells residing in large numbers in the gut. We recently published data which provides important insights into the mechanisms of immune responses against pathogenic antigens in the intestine. Therefore, we generated a new transgenic mouse model in which gut inflammation was triggered by overexpression of a single foreign antigen, influenza hemagglutinin (HA), in enterocytes (Gut 2005). Crossing these mice with transgenic animals that expressed $\alpha\beta$-T cells specific for HA (TCR-HA for CD4+ T cells and CL4-TCR for CD8+ T cells) resulted in an animal with autoreactive T cells that recognized hemagglutinin restricted to the gut. We demonstrated that in these animals the expression of a hemagglutinin on enterocytes is sufficient to trigger the development of a very mild autoimmune colitis. However, the inflammation we found was far less severe than in other models, suggesting that inflammation is partially controlled by regulatory mechanisms. FACS analysis of transgenic CD4+ and CD8+ T cells revealed an increase of Foxp3+ T cells in the mesenteric lymphnode of VILLIN-HA/TCR-HA and VILLIN-HA/CL4-TCR double transgenic mice (Gut 2005, Gastroenterology 2006, PLoS Biology, 2007). In our current work we are focusing on the origin of phenotype of pathogen-specific T cells.
Are these thymic Tregs or Tregs induced locally by high levels of IL-10? Local intestinal DCs are a potential source of IL-10 and may induce the development of IL-10 secreting T cells. What is the role of mature and immature DCs in the transgenic animal model for the induction and control of inflammation? Likewise intestinal epithelial cells (IECs) are involved in the induction of innate immune responses, particularly in the regulation of uncontrolled T cells responses by secreting cytokines. Is this regulatory network in our transgenic mouse model driven by persistent antigen and what is the role of gut epithelial cells in maintaining local Tregs?

ROLE OF COMMENSAL GUT BACTERIA AND INNATE IMMUNITY IN GASTROINTESTINAL INFLAMMATION

Markus M. Heimesaat[1], A. Fischer[1], M. Blaut[2], O. Liesenfeld[1], R. R. Schumann[1], U. B. Göbel[1], S. Bereswill[1]

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Gastrointestinal inflammation is accompanied by a shift of the dominant commensal gut microflora towards bacteria such as *Escherichia coli* and *Bacteroides/Prevotella* spp., which might aggravate intestinal inflammation via LPS or other bacterial ligands and Toll-like-receptor (TLR) signaling. We investigated gut flora changes and a possible role of innate immunity in ileitis and colitis by using mice lacking TLR 2 and 4, or the signaling adapter proteins MyD88 and TRIF.

In experimental colitis, *E. coli* increased by four orders of magnitude. Most strikingly, mice lacking TLRs 2 or 4 displayed less clinical signs of colitis, as compared to wild-type animals. This was independently confirmed by reduced clinical scores in mice lacking both, TLR2 and TLR4. Moreover, reduced colitis symptoms in $TLR2^{-/-}$, $TLR4^{-/-}$, $TLR2/4^{-/-}$ mice were associated with lower *E. coli* abundance, indicating that the *E. coli* load correlates with colitis severity.

In experimental ileitis, *E. coli* increased by nine orders of magnitude. Mice with impaired in TLR4 or MyD88 expression, but intact TRIF or TLR2 signaling, displayed reduced mortality and diminished small intestinal immunopathology. Decreased IFN-γ- and NO-levels in the inflamed ileum of TLR4-deficient mice indicated that TLR4-signaling aggravates ileitis via local mediator release from immune cells. *E. coli* strains isolated from the inflamed ileum induced TLR4-dependent NF-κB activation and nitric oxide (NO) production in HEK293 cells and peritoneal macrophages. Gnotobiotic mice treated with *E. coli* lipid A or colonized with live *E. coli* cells showed severe ileitis, whereas animals lacking TLR4 were protected. Treatment with the LPS scavenger polymyxin B ameliorated the clinical course ileitis, indicating that application of TLR-antagonists may represent a novel intervention strategy for prophylaxis and/or therapy of intestinal inflammation.

We conclude that intestinal inflammation is accompanied by a shift in the intestinal flora towards pro-inflammatory bacteria, which potentiates colitis and ileitis via TLR signalling. This underlines the role of the innate immune system as a key player in bacteria-mediated acute gastrointestinal immunopathology. The ileitis model, characterized by a pronounced shift of the gut flora towards pro-inflammatory bacteria, seems to be excellently suited to study bacteria-host interactions in acute small intestinal inflammation and to investigate novel therapies based on gut flora modulation or anti-TLR strategies.

BACTERIAL TRANSLOCATION AND RESPONSE OF MESENTERIC PREADIPOCYTES TO BACTERIAL ANTIGENS

[1] Batra, Arvind; [2] Heimesaat, Markus M.; [2] Bereswill, Stefan; [2] Fischer, Andre; [2] Plickert, Rita; [1] Pietsch, Jeannette; [1] Stroh, Thorsten; [1] Glauben, Rainer; [1] Fedke, Inka; [1] Zeitz, Martin; [1] Siegmund, Britta

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In Crohn's disease colonic infiltration is often accompanied by a hypertrophy of mesenteric fat wrapping around the inflamed intestine. The biological significance of this phenomenon is not understood yet. However, in the recent years it became evident that adipose tissue is a potent producer of various immune regulating mediators including leptin, IL-6 and TNFα. In the past we could demonstrate expression of Toll-like receptors (TLR) on murine adipocytes and preadipocytes and an increased cytokine production by these cells following TLR-specific stimulation.

To assess the extend of bacterial translocation during colitis, and hence the possibility for the direct contact between antigens from intestinal flora and mesenteric fat, models of intestinal inflammation were employed in mice, and bacterial translocation was assessed by analysis for the presence of life bacteria.

Additionally, to transfer our initial findings made in the murine system, expression of TLR in human preadipocytes initially isolated from mesenteric fat was tested by RT-PCR and TLR-specific stimulation.

While in the model of acute DSS-induced colitis no bacterial translocation occurred, in chronic DSS-induced colitis, and in *Toxoplasma gondii* induced Ileitis bacterial translocation to various mesenteric tissues including lymph nodes and fat was evident. Interestingly, in mice deficient for the TLR-adaptor protein MyD88, bacterial translocation was increased. In concordance with our previous reports in the murine system, in human preadipocytes TLR mRNA is expressed, and the cells respond to TLR-specific stimulation by increased IL-6 production.

Our data provide strong evidence, that bacterial translocation is common during ongoing intestinal inflammation. Since functional TLR are expressed on preadipocytes and adipocytes across species, the leakage of bacteria from the intestine to mesenteric fat could subsequently increase the local cytokine production of the adipose tissue. An effect, that might contribute to the mesenteric fat hypertrophy seen in Crohn's disease, and possibly even affects the initial inflammation.

PROBIOTIC-DERIVED SOLUBLE PROTEINS PREVENT INTESTINAL EPITHELIAL CELL APOPTOSIS

David Brent Polk

Department of Pediatrics, Division of Gastroenterology and Nutrition, Nashville, TN, USA

Lactobacillus rhamnosus GG (LGG) is one of the best-studied probiotic bacteria in clinical trials for treating and/or preventing several intestinal disorders, including inflammatory bowel disease (IBD). However, the clinical application of LGG and other probiotics has been limited by the paucity of information regarding their mechanisms of action.

Probiotics have been proposed to work through several mechanisms including improved barrier function, anti-bacterial, and anti-inflammatory activities; thus we have used LGG as a model probiotic to determine mechanisms of regulatory action on intestinal epithelial cells. We have reported that LGG and LGG-secreted proteins (p40 and p75) prevent cytokine-induced apoptosis in mouse & human colon epithelial cell lines by promoting the anti-apoptotic phosphatidylinositol (PI) 3-kinase-dependent activation of Akt/PKB. We have used genetic and pharmacological approaches, both *in vitro* and *in vivo* and find that EGFR kinase activity is required for p40 regulated intestinal epithelial cell anti-apoptotic response and that p40 protects the intestinal epithelium from DSS-induced tissue damage and colitis in an EGFR-dependent manner. These novel findings provide insights into the mechanisms by which probiotic LGG-derived soluble proteins regulate intestinal epithelial cell homeostasis through activating EGF receptor-dependent signaling. Therefore, our current findings identify a novel mechanism of action and provide a clinical rationale for therapeutic application of probiotic-derived soluble proteins in intestinal inflammatory disorders with increased epithelial cell apoptosis.

PROBIOTIC E. COLI (SYMBIOFLOR®2) TREATMENT MEDIATES ANTIMICROBIAL β-DEFENSIN (hBD-2) SYNTHESIS AND FECAL EXCRETION IN HUMANS

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Background. Antimicrobial defensins play an important role in host defense and seem to be a critical factor in the pathogenesis of Crohns disease. Besides direct defense at mucosal surfaces, it has been shown that defensins also regulate the composition of luminal bacteria. Probiotic bacteria such as E. coli Nissle 1917 as well as Lactobacilli stimulate the intestinal innate defense by specifically inducing antimicrobial defensin (hBD-2) in colonic epithelial cells. However, the relevance of this in vitro effect has not yet been shown in vivo. In this study we aimed to assess hBD-2 secretion into the feces after probiotic treatment of healthy volunteers.

Methods. Symbioflor® 2, a mixture of viable bacteria comprising at least 4 genotypes of one commensal strain of E. coli was administered for 21 days to 34 healthy individuals (treated n=29 and placebo n=5). HBD-2 protein expression was determined from fecal samples collected on days 0, 21 and 95 by a specific ELISA against hBD-2. To determine which E. coli genotype of the probiotic preparation mediated this effect, we performed time- (3, 6, 9 and 12h) and dose- ($1x10^8$ up to $1x10^9$ bacteria cells/ml) experiments with colonic epithelial cells in vitro. The colony forming units of fecal intestinal bacteria isolates were assessed to evaluate possible functional effects on the microbiota.

Results. 79 % (24/29) of individuals ($p<0.001$) responded with a 3.2 fold increased fecal hBD-2 peptide secretion detected after 3 weeks of treatment with Symbioflor® 2. As compared to day 0, the mean fecal hBD-2 peptide level was still elevated 10 weeks after probiotic treatment was stopped ($p<0.01$). In vitro studies revealed that only one out of four E. coli genotypes ($p<0.01$) consistently induced hBD-2 in a time- and dose-dependent manner (maximal with 12 h of incubation and 6×10^8 bacteria cells/ml ($p<0.05$)). The induction level was comparable to the positive control E. coli Nissle 1917. The other three tested genotypes which were part of the probiotic mixture did not stimulate hBD-2 mRNA expression at any dose or any measured time point. Consistent with the known regulatory effect of defensins on the flora, changes in the composition of the fecal microbiota after three weeks of patients were seen in three out of three patients.

Conclusion. This is the first study which shows an increased secretion of hBD-2 peptide into the feces upon treatment with probiotic bacteria. These in vivo- together with previously published in vitro data provide strong evidence that the specific up-regulation of host defense molecules such as human beta defensin 2 is a common mechanism of probiotic treatment.

THE EFFECT OF CONSUMPTION OF FERMENTED MILK CONTAINING *LACTOBACILLUS CASEI* SHIROTA ON INNATE IMMUNE FUNCTIONS IN HEALTHY MEN WITH A LOW NATURAL KILLER CELL ACTIVITY

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A potential beneficial aspect of probiotic food is the modulation of the immune system. In healthy individuals contradictory results exist about whether functions of the innate immunity can be modulated by probiotic bacteria. Several studies suggest that in particular healthy individuals with a low activity of natural killer (NK) cells can benefit from supplementation with probiotic bacteria.
Therefore, functions of the innate immune system of 68 healthy men with low NK cell activity were investigated in a randomized double-blind, placebo-controlled intervention study (study period 8 weeks, including a 2 weeks run-in period, 4 weeks supplementation and a 2 weeks follow-up period) by flow cytometry. Individuals of the verum group (n=34) received a milk drink containing the probiotic *Lactobacillus casei* Shirota (LcS; $1{,}95 \times 10^{10}$ cfu/day) during the 4 weeks of supplementation. Individuals of the control group (n=34) consumed the milk drink without probiotic bacteria. The intake of LcS resulted in no significant change of the phagocytic activity and -intensity of monocytes and neutrophils in the blood. In addition, there was no effect on the oxidative burst, which was also assessed by activity and intensity. The proportion of monocytes and neutrophils in the blood remained the same during the whole study period. The intake of LcS also did not affect NK cell activity in the blood.
In summary, the innate immune functions examined in this trial cannot be modulated by supplementation with the probiotic LcS in this study group with a low NK cell activity. However, dose-response-relationships cannot be ruled out as other trials than the present study often used higher LcS dosages. Dietary habits of study subjects may also be relevant for the outcome of the trial, which in open studies may impede to demonstrate NK cell-specific modulatory activities of probiotics.

A PROTEASOME INHIBITOR SECRETED BY PROBIOTIC BACTERIA BLOCKS INFLAMMATORY SIGNALING

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Scientific Interests and available Modelsystems:

impact of the endogenous flora and atypical mycobacteria on immunity, proteasome inhibitors released by probiotic bacteria, proteasomes dependent inflammatory signaling. Models: infection models, germfree mice, proteasomes inhibitors, animal models for colitis.

A proteasome inhibitor secreted by probiotic bacteria inhibits inflammatory signal pathways.

Proteolysis regulates many intracellular processes and is important in maintaining biological homeostasis. The major component of cytoplasmic protein degradation is the proteasome. Pharmacological inhibition of the proteasome activity has been shown to be an effective and suitable therapeutic treatment for inflammatory and immune disorders.

Dysregulation of the proteasomal protein processing pathway is associated with numerous inherited and acquired diseases including asthma, Alzheimer´s disease, several types of cancers, autoimmune thyroid disease, inflammatory bowel disease and many others. The therapeutic use of different proteasome inhibitors alone and in combination with other drugs has been shown to be shown to be effective treatment in several diseases. We found that probiotic bacteria (VSL#3) secrete a proteasome inhibitor (I-VSL#3) that efficiently blocks the chymotryptic activity of immunoproteasomes (I-proteasomes) which is increased in many inflammatory conditions. Accordingly, I-VSL#3 was able to greatly inhibit the NF-κB mediated signalling by down-regulating the processing and degradation of the NF-κB p50 precursor p105 and IκBα, respectively. Further, I-VSL#3 was also able to dramatically block the activation of the TPL-2 /ERK MAP kinase pathway, demonstrating its anti-inflammatory potential.

SUCCESSFUL THERAPY OF UNSPECIFIC PROLONGED DIARRHOEA IN INFANTS AND TODDLERS WITH THE PROBIOTIC *E. COLI* NISSLE 1917

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Background. Infants and toddlers with prolonged diarrhoea over several days are in danger of developing dehydration and an acute deterioration of their general state of health. So far no effective causal therapy exists. Therefore in a confirmatory, randomized, double-blind clinical trial the efficacy of the probiotic bacterial strain *E. coli* Nissle 1917 (EcN) as compared to a placebo was tested.

Patients and Methods: In total, 151 children aged 1 month to 47 months (Ø 25 months) with unspecific prolonged diarrhoea (> 3 watery or loose stools without blood in 24 hours of a diarrhoeal episode, which has been persisting for more than 4 consecutive days but not longer than 14 days) were randomized in a double-blind design to receive either the probiotic EcN suspension (n = 75) or placebo (n = 76). All children were dehydrated to a medium extent (5 - 10% loss of body weight) and received 1 to 3 ml verum or placebo suspension orally per day depending on the age (1 ml suspension contained 10^8 viable EcN bacteria) for 21 days. At study commencement, rehydration (ORL according to WHO) was performed once.

Results. The number of patients showing a reduction of stool frequency to less than 3 watery or loose stools in 24 hours over a period of at least 4 consecutive days (response rate) was higher in the EcN group than in the placebo group already on day 7 (EcN 78.7%, placebo 59.2%) The response rate increased continuously as was measured on days 14 (EcN 93.3%, placebo 65.8%) and 21 (EcN 98.7%, placebo 71.1%). The two-sample test of rates following a group sequential test design showed statistically significant superiority for EcN on both days 14 ($p = 0.0017$) and 21 ($p<0.0001$).

Conclusion. Prolonged diarrhoea can be successfully treated. Due to its excellent efficacy the probiotic EcN is a suitable remedy.

Keywords. prolonged diarrhoea, infants, probiotic, Escherichia coli Nissle 1917

INVESTIGATION OF THE ANTIMUTAGENIC ACTIVITY OF PROBIOTIC *E. COLI* NISSLE 1917 AGAINST 4-NITROQUINOLINE-1-OXIDE

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Background. Probiotics are viable, non-pathogenic microorganisms which when administered in adequate amount confer health benefits on the host. Some probiotics, notably strains of grampositive lactobacilli and bifidobacteria (1), have been shown to prevent the action of mutagenic or carcinogenic substances. Here, we investigated, whether the gramnegative probiotic *E. coli* Nissle 1917 (EcN) might also exhibit antimutagenic activities.

Methods. Two different mutagenicity tests were employed, the classical Ames-test (2) using specific *Salmonella* and *E. coli* mutant strains, and the Comet-assay (3) using Caco-2 epithelial cells. 4-nitroquinoline-1-oxide (NQO) was used as the mutagenic substance. For both mutagenicity tests, NQO was co-incubated with either live or heat-killed EcN bacteria or with sterile filtered spent supernatants of EcN cultures.

NQO in saline served as positive control. After separation from bacterial cells by centrifugation and sterile filtration, the co-incubation mixtures were tested for mutagenic activity.

Results. EcN itself did not exhibit any mutagenic potential. Mutagenic activity of NQO declined depending on the amount of live EcN bacteria present in the assay, whereas heat-killed EcN and cell-free supernatants had no effect. The antimutagenic action of live EcN bacteria correlated with a shift in the absorption spectrum of NQO as shown by UV/VIS spectroscopy, suggesting EcN-mediated transformation of the NQO molecule.

Conclusion. Here, we could show for the first time that live *E. coli* Nissle 1917 bacteria display antimutagenic action against the mutagenic substance NQO. This novel activity underlines the beneficial nature of *E. coli* Nissle 1917.

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EFFECTS OF PROBIOTICS AND/OR IMMUNO-NUTRITION ON THE IMMUNE SYSTEM AND THE ONE-YEAR SURVIVAL OF ELDERLY PATIENTS WITH PROXIMAL FEMUR FRACTURES

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Scientific Interests:
Immunity and Bone/Wound Healing in Elderly Patients

Malnutrition is a common cause of secondary immunodeficiency. Especially, the already altered immune status in the elderly can be further affected by nutritional defects. After an accident with trauma or after substantial operations an inflammatory response is elicited with liberation of pro-inflammatory factors and the formation of free radicals that are partly responsible for the poor outcome. Moreover, it is relatively frequent that this population has extended hospital stays. Taking all this into account it is easy to understand that the elderly patients in this setting are exposed to severe complications such as thromboembolism, cardiac ischemic disease, or exacerbations of chronic conditions, and are also extremely vulnerable to wound infections, pneumonia, and urine tract infection.

Recent studies have clearly shown that the intestinal flora has a great impact on the immune system and that the intake of probiotics or immune nutrition improves the immune system and subsequently reduce the infection rate in vulnerable patients. In the same line of evidence, it was clearly demonstrated that administration of probiotics improves the clinical outcome in patients after extended pancreas operations. We hypothesize that the improvement of the immunological status leads to a better inflammatory response status, a lower rate of infections and to a subsequent reduction in mortality. The *primary objective* is to assess the one year survival rate after surgical intervention in patients older than 65 years with proximal femur fracture, further treated with oral probiotics and/or immunonutrition in comparison to placebo. The *secondary objectives* are to assess the influence in the infection rate and in the parameters related to inflammatory response and immune system.

VSL#3 ATTENUATES INFLAMMATORY GENE EXPRESSION IN THE CECUM OF IL10KO MICE

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VSL#3 is a high concentration probiotic of eight live freeze- dried bacterial species, which are normal colonisers of the human intestinal tract. VSL#3 is thought to be of potential benefit to individuals with mild to moderate colitis and in the prevention of the onset of acute pouchitis.

This study aims to investigate the therapeutic effect of VSL#3 in IL10 knockout mice. IL10 knockout mice develop spontaneous colitis and are therefore a suitable animal model for the study of probiotic action. Wildtype and IL10 knockout mice were fed with placebo or VSL#3 for 24 weeks. Histolopathological analysis identified some beneficial effect of VSL#3 in the cecum but not in the distal colon. Using real time PCR the therapeutic action of VSL#3 in both the cecum and ascending colon of the IL10 knockout mice was investigated. The pro-inflammatory genes CXCL9, CXCL10, TNF and S100A8 were selected following a detailed affymetrix microarray analysis of the colons of wildtype and IL10 knockout mice. TNF and the TNF-induced chemokines CXCL9, CXCL10, as well as the inflammatory marker S100A8, all showed highly significant foldchanges identifying them as ideal gene candidates to monitor both the severity of inflammation and the potential therapeutic action of VSL#3. Of the 3 housekeeping genes tested (beta Actin, HPRT1, 18S), 18S showed the least variation across all treatments and was therefore used for normalisation of the real time data. The log normalised data was analysed in Minitab using the Mann-Whitney test. Consistent with the histopathology data the probiotic VSL#3 was shown to reduce pro-inflammatory gene expression of CXCL9 (3 fold, P=0.0149), CXCL10 (2.9 fold, P=0.0043) and TNF (2 fold, P=0.0262) in the cecum but not in the ascending colon of the IL10 knockout mice.

PROBIOTICS IN ACUTE PANCREATITS: DESIGN AND OUTCOME OF A MULTICENTER TRIAL

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Acute pancreatitis (AP) is an acute inflammatory disease caused by either gall stones or excessive alcohol abuse. AP usually runs a mild, self-limiting cause, but about a fifth of patients will develop necrotising pancreatitis, which is associated with a 10–30% mortality rate. Mortality is mostly attributed to infectious complications and infection of (peri)pancreatic necrotic tissue in particular. The infections are thought to be the sequelae of a cascade of events which starts with small-bowel bacterial overgrowth, mucosal barrier failure, and a proinflammatory response leading to translocation of intestinal bacteria. Antibiotic prophylaxis has been shown to be ineffective in preventing these infections. Two small studies with probiotics however did indicate a potential to reduce the infectious complications of this disease.

The prospects of probiotic therapy in AP were based on their ability to inhibit the growth of pathogens. We have selected the probiotic strains which were best capable to inhibit clinical isolates from AP patients. Probiotics also have the ability to improve gut barrier function and the combination of selected strains was very effective in a rat model of AP to prevent the breakdown of gutbarrier function. Since AP is an acute inflammatory disease, probiotics were selected which could downregulate pro-inflammatory cytokines. Based on these in vitro and in vivo data, a multispecies mixture of probiotic bacteria was composed and tested in the in vivo rat model for AP. In rats, the probiotics reduced gut mucosal damage, pancreas pathology and improved survival rates.

The Dutch Acute Pancreatitis Study Group has initiated a multicenter study (PROPATRIA) on the effectiveness of this multispecies probiotics on prevention of infectious complications in AP. The placebo group was treated with high fiber, high calory enteral nutrition administered trough a nasogastric tube. The probiotics group received the enteral nutrition as well as a daily dose of a 10^{10} probiotic bacteria: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium bifidum*, and *Bifidobacterium lactis* for a period of maximal 28 days.

This trial in patients with predicted severe acute pancreatitis, in which a total of 298 patients were included, showed no beneficial effect of probiotic prophylaxis on the occurrence of infectious complications. However, mortality in the probiotics group was about twice as high as in the placebo group (24 (16%) and 9 (6%), respectively, p 0.01). In 8 patients in the probiotics group (none in the placebo group) bowel ischaemia was the cause of death. Thus, this combination of probiotics should not be administered routinely in patients with predicted severe acute pancreatitis, and such preparations can no longer be considered to be harmless adjuncts to enteral nutrition.

ROLE OF NF-κB ACTIVATION IN INTESTINAL EPITHELIAL CELLS IN INTESTINAL IMMUNE HOMEOSTASIS

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Scientific Interests and available Modelsystems:

Although the intestine contains billions of bacteria that can be recognized by Toll-like Receptors (TLRs), the mucosal immune system stays hyporesponsive towards the gut microflora. Intestinal epithelial cells (IEC) form a physical barrier between the gut lumen and the mucosa, which prevents the interaction of microflora with mucosal immune cells. Disruption of the epithelial barrier and subsequent immune responses to the microflora are thought to be key factors in the development of Inflammatory Bowel Diseases (IBD). In our lab we are investigating the role of NF-κB activation in IECs in intestinal immune homeostasis by means of conditional gene targeting in mice. For this purpose, we use mice with deletion of NEMO specifically in intestinal epithelial cells ($NEMO^{IEC\text{-}KO}$), which spontaneously develop severe colitis.

We recently showed that activation of the transcription factor NF-κB is essential for maintaining the integrity of the intestinal epithelial barrier. Activation of NF-κB is crucially dependent on NEMO, an essential component of the IKK complex. $NEMO^{IEC\text{-}KO}$ mice show increased apoptosis of intestinal epithelial cells, disruption of the epithelial barrier and subsequent translocation of bacteria into the mucosa, leading to severe colon inflammation. Genetic deficiency of MyD88, an essential adapter for TLR-induced signalling, rescues $NEMO^{IEC\text{-}KO}$ mice from colonic inflammation, suggesting that bacteria triggering TLR signalling are important for the development of inflammation in these mice. In order to evaluate the causative role of the microflora in this novel mouse model of IBD, we are investigating intestinal immune homeostasis in $NEMO^{IEC\text{-}KO}$ mice raised in a germ-free environment. We are using these germ-free $NEMO^{IEC\text{-}KO}$ mice to elucidate the role of bacteria and the cellular specificity of TLR signalling in the development of intestinal inflammation in $NEMO^{IEC\text{-}KO}$ mice.

THE ROLE OF INTESTINAL EPITHELIA FOR THE INNATE IMMUNE RESPONSE TO NON-INVASIVE FACULTATIVE PATHOGENS

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Scientific Interests:
epithelial barrier function, IBD, bacterial oncogenes

Available Modelsystems:
polarized IEC monolayers (PTK6 and MDCK cells), *in vitro Helicobacter hepaticus* infection

Intestinal epithelial cells (IEC) form a barrier to prevent microbial factors from entering host organisms. A defective barrier exposes non-invasive facultative pathogens to the immune system contributing to inflammation of the intestine, which can manifest as Inflammatory Bowel Disease (IBD). Bacterial adhesion to and invasion of IEC is increased in IBD or in IBD mouse models. In our experiments we tested if a barrier defect of the IEC promotes adhesion and invasion of IEC with otherwise non-invasive facultative pathogens. Therefore, we infected polarized epithelial cells (MDCK or PTK6 cells) with the non-invasive facultative pathogen *Helicobacter hepaticus*. We compared infection from apical and basal-lateral with intact barrier and polarized epithelia after opening of their barrier briefly via Ca^{++}-depletion.

In apically infected polarized epithelia almost no bacteria adhere after 18 hours when the barrier is intact. In contrast, infection of cell monolayers with open barrier or from basal-lateral lead to adhesion of 133 bacteria/100 IEC or 112 bacteria/100 IEC after 18 hours, respectively. Adhesion was followed by invasion as early as 2 hours after start of infection. Sixteen hours later, 70 H. hepaticus/100 IEC were intracellular compared to 133 attached bacteria/100 IEC. Immunofluorescence stainings for various cellular organelles revealed that intracellular *H. hepaticus* are incorporated into lysosomes. However, preliminary data of gentamycin protection assays suggest that H. hepaticus can overcome lysosomal degradation and is able to replicate intracellular in IEC. Future experiments are aimed at clarifying how H. hepaticus overcomes intracellular degradation, its role for IBD-like inflammation of the colon in mice and if the basal-lateral adhesion and uptake of bacteria is part of a physiological host response of IEC as part of the general innate immune response.

DNA ARRAY ANALYSIS OF T84 EPITHELIAL CELLS CO-INCUBATED WITH GRAM-NEGATIVE OR GRAM-POSITIVE PROBIOTIC BACTERIA: NEW PERSPECTIVES FOR INFLAMMATORY BOWEL DISEASE TREATMENT

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The gastrointestinal tract harbours a complex microbial ecosystem, engaged in a continuous crosstalk with the host. The balanced relationship between intestinal epithelial cells (IECs, model = T84) and gut microbes can be disturbed, resulting in activation of the mucosal immune system to the resident microflora contributing to inflammatory bowel diseases (IBD).

Using a polarized T84 cell culture model the cellular responses of IECs after contact with bacteria were analyzed. For mRNA profiling of IECs Affymetrix microarrays were employed. Probiotic *E. coli* Nissle 1917 (EcN) strain or *Lactobacilli* reinforce the barrier function and adjust the synthesis of proinflammatory cytokines. The enteropathogenic reference strain *E. coli E2348/69* (EPEC) disrupts the barrier function and activates the IκB/NF-κB pathway.

Based on more than 300 differentially regulated genes and taking into account the fact that IBD may develop after defects of barrier function, we focussed on genes encoding tight junction (ZO-2) and adherence junction (beta-catenin and E-cadherin) proteins. Characterized genes were verified by qRT-PCR and immunohistochemistry. Barrier function of epithelial cells appeared to be modulated by different protein kinase C (PKC) isoforms: atypical PKCζ leads to the disruption of tight junctions after EPEC infection. Our miro-array data indicate that novel PKCδ and especially conventional PKCα are engaged in the reinforcement of barrier function after co-incubation with EcN. E-cadherin co-localizes with novel PKCε in response to gram-positive *Lactobacillus fermentum*. In general, regulation of barrier function is subject to the activity of different PKC isoforms depending on the impact of different probiotic bacteria (gram-positive or gram-negative).

This study revealed cellular responses of IECs specifically induced by the probiotic *E. coli* Nissle 1917 or *Lactobacilli*. Further insight into the underlying molecular mechanisms will foster the development of improved strategies for the treatment of gastrointestinal diseases including IBD.

ENTEROCOCCUS FAECALIS METALLOPROTEASE CONTRIBUTES TO THE DEVELOPMENT OF CHRONIC INTESTINAL INFLAMMATION THROUGH IMPAIRMENT OF EPITHELIAL BARRIER FUNCTION

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Commensal bacteria like *Enterococcus faecalis* are able to produce proteases which may play a critical role for the development of chronic intestinal inflammation and inflammatory bowel disease. *In vivo* studies showed that IL-10-deficient mice (129SvEv) monoassociated with *E. faecalis* strain OG1RF, which produces a metalloprotease (GelE) and a serinprotease (SprE), develop severe colitis after 15 weeks. In contrast monoassociation with the isogenic mutant for gelatinase (strain TX5264) and for serinprotease (strain TX5243) significantly decreased inflammation demonstrating that *E. faecalis* GelE and SprE contribute to colitis development in IL-10-/- mice.

In order to investigate the impact of these proteases *in vitro* we used the intestinal epithelial cell line T84. T84 colonocytes develop a transepithelial electrical resistance (TEER), which is measured to monitor the integrity of the epithelial barrier. Apical stimulation of T84 cells with concentrated bacteria conditioned media (CM) of *E. faecalis* OG1RF containing GelE and SprE decreased TEER to approximately 25% of controls after 24h. This effect could not be observed with the concentrated CM of the isogenic *gelE* and *sprE* mutants. The gelatinase of *E. faecalis* OG1RF was purified by fast protein liquid chromatography using an anion exchanger (Hi Load 16/10 Sepharose Q High Performance). The stimulation with gelatinase alone had no influence on TEER of T84 cells whereas in combination with the concentrated CM of *gelE* mutant, that also contains SprE, the barrier integrity was significantly reduced.

The reduction of TEER suggests that GelE and SprE enhance inflammation through the impairment of epithelial barrier integrity. Our results indicate that experimental colitis is not only triggered by bacteria, but also strain by specific virulence factors like proteases.

POST-TRANSLATIONAL INHIBITION OF IP-10 PROTEIN SECRETION IN INTESTINAL EPITHELIAL CELLS THROUGH UBIQUITIN-MEDIATED MECHANISMS: BACTERIAL STRAIN-SPECIFIC EFFECTS OF VSL#3

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Background. Clinical and experimental studies suggest that the probiotic mixture VSL#3 has protective activities in the context of inflammatory bowel disease (IBD) and animal models of colitis. The aim of the study was to reveal bacterial strain-specific mechanisms underlying the anti-inflammatory potential of VSL#3 in primary and intestinal epithelial cell (IEC) lines.

Results. VSL#3 inhibited TNF-induced protein secretion of the T-cell chemokine interferon-inducible protein (IP-10) in Mode-K cells. Protease- and heat-sensitive cell surface structures of *L.casei* were identified as active anti-inflammatory component of the probiotic mixture. Interestingly, *L.casei* failed to inhibit TNF-induced NFκB RelA (p65) recruitment to the IP-10 promoter as well as TNF-induced IP-10 mRNA and IP-10-specific reporter gene expression, suggesting post-transcriptional inhibitory mechanisms. Although *L.casei* triggered TNF-induced activation of the translational initiation machinery including, EIF4G and EIF4E, the intracellular accumulation of IP-10 protein was only transient. Co-immunoprecipitation analysis showed that *L.casei* induces selective ubiquitination of IP-10, supporting subsequent proteasomal degradation of this chemokine in IEC. IP-10 overexpression experiments revealed TNF stimulus-independent mechanisms for the inhibition of IP-10 expression. Finally, VSL#3 and *L.casei* failed to attenuate IP-10 protein expression in primary ileal epithelial cells and the development of tissue pathology in the ileum of heterozygous $TNF^{\Delta ARE}$/WT mice.

Conclusion. *L.casei* was identified as active component of VSL#3 targeting IP-10 secretion through ubiquitin-mediated inhibitory mechanisms. Feeding experiments in $TNF^{\Delta ARE}$ mice failed to demonstrate protective effects of VSL#3 and *L.casei* in an animal model of chronic ileitis, suggesting the colon as primary target of probiotic intervention.

THE ANTI-INFLAMMATORY EFFECTS OF PROBIOTIC DNA SEEM TO BE MEDIATED BY TLR9 IN ENTEROCYTES

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Scientific Interests and available Modelsystems:

Molecular biology, gene and protein expression profiling of TLRs systems, intercellular signalling pathway and microbiology. In vitro cell and tissue culture models.

Background & Aims. The intestinal immunosystem distinguishes prokaryotic DNA from vertebrate DNA by detecting CpG motifs which are abundant and unmethylated in prokaryotics. We tested whether the anti-inflammatory effects of probiotic bacteria are due to their genomic DNA, whether expression of TLR9 mediates this effects and whether TLR9 silencing disturbs this effects.

Methods. Genomic DNA from probiotic bacteria, DNA from calf thymus or CpG-ODN were incubated with human colonic epithelial cells lines for 1- 48 h. TLR9 expression was assessed before and after TLR9 silencing and stimulation with probiotic DNA, Calf thymus DNA and two different ODN sequences.TLR9 mRNA and protein expressions were assessed by TaqMan RT-PCR and western blot, respectively. Secreted IL-8 was determined using ELISA.

Results. The probiotic DNA reduced IL-8 secretion by 33±2, 1 %, which is a key pro-inflammatory cytokine, in intestinal inflammation disease, whereas calf thymus DNA and ODNs had no effect. The regulation was dose and time dependent.TLR9 silencing abolished these effects.

Conclusion. These results indicate that TLR9 expression is essential in mediating the anti-inflammatory effects of probiotic DNA.

CD4 T CELL SUBSETS AND RESPONSES INCLUDING TREG AND TH17 CELLS

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Pathogen associated molecular patterns (PAMPs), the cytokine milieu, antigen dose and affinity, and the presence of costimulatory and adhesion molecules play a critical role in determining the nature of T cell responses. Dependent upon the context and environment in which antigen is presented, naïve $CD4^+$ T cells can differentiate into: IFN-γ and TNF-α-producing Th1 cells; IL-4, IL-5, IL-10 and IL-13-producing Th2 cells; IL-17A, IL-17F and IL-22-producing Th17 cells; or an array of inducible regulatory T cell (Treg) subsets. Such specific cell subsets have presumably evolved to more effectively counter infections by diverse pathogens, or to regulate anti-self responses and limit immune pathology. The factors and conditions determining differentiation of the various T cell subsets and their fate and function with particular emphasis on Treg and Th17 cells and will summarized in my presentation at the meeting in Seeon.

COMPERATIVE GENOMIC ANALYSIS FOR THE PRESENCE OF POTENTIAL ENTEROCOCCAL VIRULENCE FACTORS OF THE PROBIOTIC *ENTEROCOCCUS FAECALIS* SYMBIOFLOR 1

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Scientific Interests and available Modelsystems:
Molecular biology, cellular microbiology, genomics, microarrays, bioinformatics / genetic engeneering of bacteria, cell culture, animal infection, *Galleria mellonella* infection model

Enterococci are members of the natural microbiota of animal and human intestinal tracts and are capable of causing opportunistic infections. They are also used as starter cultures in the food industry as well as in health supplements and probiotics by the pharmaceutical industry. This Janus-faced status requires a careful evaluation on the basis of pathogenic traits to ensure the safety of the strain used to produce food and pharmaceuticals. We performed gapped-genome sequencing of a probiotic strain *E. faecalis* Symbioflor 1 and present initial results deriving from comparative genome analysis with that of the previously sequenced pathogenic clinical isolate *E. faecalis* V583. There was strong overall conservation of synteny between both strains and a detailed analysis revealed the absence of large genomic regions from the chromosome of the probiotic strain, indicating gene loss. Genes absent from the Symbioflor 1 strain included those encoding the enterococcal cytolysin, enterococcal surface protein, and gelatinase (coccolysin) as well as hyaluronidase and the peptide antibiotic AS-48. This data was confirmed using PCR primers specific for the respective genes. However, other enterococcal determinants such as aggregation substance, collagen adhesion protein, the ability to resist oxygen anions as well as capsule formation were detected. The presence of these traits may be advantageous for the strain Symbioflor 1 since they potentially enable colonisation and proliferation of the bacterium on mucosal surfaces thereby conferring on it probiotic traits.

SIMPLIFIED MODELS FOR MECHANISTIC STUDIES ON HOST-MICROBE INTERACTIONS

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Considerable efforts have been made to better define the role of the intestinal microbiota in host physiology. While the knowledge on specific functions of gut bacteria has considerably increased, very little is known whether and to which extent host and nutrition factors affect intestinal bacteria and possibly their reaction towards the host. To study the impact of host factors on gut microbes, germfree mice were mono-associated with commensal *Escherichia coli*. We analyzed the bacterial response to the conditions in the gut by two-dimensional gelelectrophoresis (2D-GE) followed by electro-spray ionization-tandem mass spectrometry (ESI-MS/MS) to identify bacterial proteins recovered from the gastrointestinal tract. Fifty proteins were identified in a set of proteins stably and differentially expressed by cecal and fecal *E. coli* in comparison with anaerobically grown *E. coli*. The ascribed protein functions suggest that the host-associated bacteria adapt their metabolism to a wider spectrum of substrates. Candidates for specific bacterial responses to the host environment are 10 identified proteins with unknown or poorly characterized physiological function and three proteins that have so far only been inferred from predictions or by homology.

These investigations will be extended to a gnotobiotic rat model developed in parallel. The rats are associated with seven dominant bacterial species from the human gut. The bacteria were chosen because of their ability to catalyze major well known processes occurring in the colon and the availability of their genome sequences. The selected species form a stable community in the gnotobiotic animals as evident from microbial analyses of intestinal contents obtained from various gut sections. We will use this model to study by proteomics the influence of dietary and host factors on the model community and we will validate the results by comparing the data with those obtained with human microbiota-associated and conventional animals.

ROLE OF GUT BACTERIA IN INTESTINAL INFLAMMATION OF THE IL-10$^{-/-}$ MOUSE

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Ulcerative colitis and Crohn's disease are relapsing, immunologically mediated disorders that are referred as inflammatory bowel disease (IBD). Data from clinical observations and animal models show that intestinal bacteria play a role in the initiation and perpetuation of chronic enterocolits and associated systemic inflammation in a genetic susceptible host and are capable to take advantage of inflammatory conditions in the gut by increased colonization. To characterize changes in the intestinal microbiota composition under inflammatory conditions and to identify bacterial species involved in chronic gut inflammation the microbiota of 1, 8, 16 and 24 week old 129-SvEv IL-10$^{-/-}$ and wild type (Wt) mice, kept under specific pathogen free conditions (SPF), was compared with culture dependent methods and with different molecular techniques. Histopathological analysis of gut tissue sections showed that IL-10$^{-/-}$ animals develop moderate inflammation of caecum and colon, which increases with age. Simultaneously, an increase of enterobacteria, bacteroides and the ruminococcus/ clostridium cluster was observed in the caecum of the knock out animals. Particularly cell numbers of enterobacteria were significantly higher in IL-10$^{-/-}$ mice compared to the Wt. This was further supported by analysis of the intestinal bacterial composition with PCR-denaturing gradient gel electrophoresis (PCR-DGGE), displaying a reduced microbial diversity of IL-10$^{-/-}$ mice in contrast to the Wt and an increase of single bacterial groups in the colon. Isolation and characterization of enterobacteria revealed that IL-10$^{-/-}$ as well as Wt mice were colonized by a single *Escherichia coli* strain. This strain belongs to the virulence associated phylogenetic group B2 and exhibits a great variety of fitness/virulence factors. In conclusion, only few bacterial species are able to deal with inflammatory conditions of the bowel. Especially group B2 *E. coli* seem to be highly adaptive, which is possibly enabled by the possession of different virulence features.

MICROBIOTA, SCFA IN LEAN AND OVERWEIGHED HEALTHY SUBJECTS

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Scientific Interests:

Human microbiotas, detection, culturing, probiotics, prebiotics

Available Modelsystems:

Probiotics, prebiotics, culturing, qPCR, biochips

Obesity has recently been linked to the human microbiota and the production of short chain fatty acids (SCFA). However, these findings rely on rather small and defined groups of volunteers or animal models. A total of 98 subjects volunteered in this study. The body mass index (BMI in kg/m^2) of 30 volunteers was in the lean range (18.5 - 24.9), 35 were overweighed (25.0 - 29.9) and 33 were obese (>30). The total amount of SCFA was higher in the obese subject group (103.87 mM, P=0.024) than in the lean subject group (84.60 mM). Proportions of the individual SCFA changed in favour of propionate in overweighed and obese subjects (P=0.035). The most abundant bacterial groups in feces of lean and obese subjects belonged to the phyla *Firmicutes* and *Bacteroidetes*. The ratio of *Firmicutes* to *Bacteroidetes* changed in favour of the *Bacteroidetes* in obese subjects (P=0.001). Obese subjects have higher SCFA concentrations than lean subjects, especially propionate while the microbiota is changed in favour of *Bacteroidetes*.

ARGD-DEPENDENT QUORUM SENSING AFFECTS BIOFILM FORMATION, VIRULENCE AND GLOBAL GENE EXPRESSION PROFILES IN *LISTERIA MONOCYTOGENES*

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Scientific Interests:
- mechanisms of anti-inflammatory effects of probiotic bifidobacteria
- quorum sensing in listeria

Autoinducing peptides are used in cell density dependent quorum sensing of Gram positive organisms. One example is the *agr* system, which is involved in virulence gene expression in staphylococci. The *agr* system is composed of a four gene operon with *agrB* involved in the proteolytic processing/export of the gene product of *agrD*, a post transitionally modified peptide, and *agrA*/*agrC* encoding for the histidine kinase/response regulator.
An operon with high structural and sequence similarity to the staphylococcal *arg* system was recently identified in the genome of *L. monocytogenes* EGDe. Here, we present the phenotypic characterisation of a deletion mutant of *L. monocytogenes* EGDe lacking the quorum sensing peptide AgrD. The Δ*argD* mutant showed a significant defect in biofilm formation. Western blot analysis of cell wall extracts showed a marked reduced expression of InlA, a major virulence factor of *L. monocytogenes* in the Δ*argD* mutant. In line with these findings, invasion of Δ*argD* into Caco-2 cells was significantly reduced compared to EGDe wt. Both biofilm formation and invasion could be complemented by single copy chromosomal integration of *argD* under the control of a constitutive promoter. Additionally, when the Δ*argD* mutant was mixed with wild type EGDe, as little as 1 % of the wild type in the inoculum was sufficient to completely restore biofilm formation of EGDe Δ*argD* to wt levels. Using bioluminescence *in vivo* imaging, a significant attenuation of EGDe Δ*argD* in a murine model of listeriosis could be shown. Moreover, microarray analysis revealed that expression of a large number of genes belonging to all functional categories was affected in EGDe Δ*argD* both in exponential and stationary growth phase indicating a global impact of *agr*-dependent quorum sensing on bacterial physiology.

GROWTH PHYSIOLOGY AND BIOSYNTHESIS OF BIOGENIC AMINES AND γ–AMINOBUTYRIC ACID BY HUMAN, COMMENSAL / PROBIOTIC STRAINS OF *ESCHERICHIA COLI*

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Commensal *Escherichia coli* is the predominant facultative anaerobe in the gastrointestinal tracts of mammals. Nutrients for growth, persistence and colonization come from ingested food, epithelial and bacterial debris, and the host mucus layer.

As a result of the anaerobic sugar utilization, pH fluctuates from 8 to 4 in its nutrient-defined niche. In order to enhance survival, most, if not all commensal and pathogenic strains of *E. coli* possess inducible stress resistance systems that collaboratively enhance fitness during stomach passage and gut colonization.

Probiotic *E. coli* strains, *e.g.* L1931, a powerful colicin producer, and Nissle 1918 have been used successfully in microbial and immunotherapy for decades. Recently, the isolation of *E. coli* L2000 announces promising prospects of new therapy approaches since effective medication of infectious colitis of apes was observed.

Both, *E. coli* L1000 and L2000, anaerobically fermented on trypton and glucose containing medium yield cell densities of about 3-4×10^8 mL^{-1}, without pH-controlling.

The proton concentration during exponential growth moves to pH 4.1, extracellular ammonium increases by 2.0 mmol/L. Under this subject to high stress, only 2 g/L glucose is metabolized. To maintain intracellular pH at a physiological level different amino-acid decarboxylase pathways are active in a pH-dependent manner. The first response to the increasing proton concentration is the formation of cadaverine from lysine, followed by decarboxylation of ornithine to putrescine and arginine to agmatine. In both strains the final part of the acid stress response regulation system is the production of 4-aminobutyrate from glutamate.

Uptake of amino acids is equimolar to excretion of their corresponding amines and the import/export happens *via* “amino acid:amine” antiporter systems in the same order for both *E. coli* L1000 and *E. coli* L2000. In contrast to *E. coli* L2000, the formation of 4-aminobutyrate seems to be accelerated and reaches higher levels in *E. coli* L1000.

INNATE SIGNALING BY INTESTINAL MICROBES AND ITS RELEVANCE TO EPITHELIAL INTEGRITY

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Increased permeability of the intestinal epithelium or ‘leaky gut’ is now recognized as having a role the pathophysiology of inflammatory bowel disease (IBD), irritable bowel disease (IBS), as well as other diseases. In IBD, altered permeability increases the infiltration of pro-inflammatory stimuli to the underlying immune cells triggering further cytokine induced changes to the tight junction and a vicious cycle of barrier dysfunction and inflammation. Consequently, modulation of epithelial permeability is an interesting target for novel therapeutic or preventative treatments against a range of diseases. The crucial structures control that hold epithelial cells together and control epithelial permeability are the tight junctions that encircle the upper part of the lateral surfaces of the adjacent epithelial cells to create "kisses" in the plasma membrane. Evidence for probiotic effects on barrier function have been demonstrated in rat models of chronic stress, hemorrhagic shock and sepsis although the mechanisms have not been fully elucidated. Evidence from in vitro studies also suggests that particular strains of probiotics can protect against barrier dysfunctions caused by invasive pathogens or pro-inflammatory cytokines. Recent data will also be presented on the modulation of epithelial integrity in humans by feeding of an intestinal Lactobacillus species. The results of this study provide a possible explanation for the reported protective effects of certain probiotic strains on barrier disruption by inflammatory cytokines, chemicals and infectious agents and have implications for the treatment and prevention of intestinal inflammation.

SELECTIVE INFLUENCE OF TCF-4 MEDIATED WNT SIGNALING ON INTESTINAL INNATE AND ADAPTIVE IMMUNITY OF ILEAL CROHN'S DISEASE

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Background. Ileal Crohn's disease (CD) is characterized by diminished antibacterial activity and a specific decrease of small intestinal Paneth cell α-defensins HD5 and 6. We previously reported a causal link between this decrease and the Wnt pathway transcription factor Tcf-4. Wnt signaling has an important function in intestinal epithelium renewal, regulating stem cell maintenance and their transition to Paneth-cells. The involvement of disturbed Wnt signaling, resulting in alleviated innate immunity, reveals a new mechanism for ileal CD pathogenesis. To further investigate the pathways influence in the disease, we aimed to assess expression of Tcf4 target and WNT pathway genes other then HD5 and 6.

Methods. RNA was isolated from ileal biopsies of healthy individuals (n=14) and CD patients (ileal CD n=28, colonic CD n=11). The mRNA levels of genes, encoding Wnt/Tcf-4 pathway factors as well as Tcf-4 target genes (β-catenin, CBP, Hic-5, ICAT, NLK, Sox-9, p53, p300 Axin-1 and -2, CDX-1, c-jun, Claudin-1, c-myc, CyclD-1, CD44, EphB-3, Ephrin-B1 and -B2, MMP-7, PPAR-δ, APC, CUL-1, DKK-1 and -2, DVL-2 and -3, Frizzled-5, Gastrin, LEF-1, LRP-5, PGLYP-1, SFRP-3, Tcf-1) were quantified using real-time PCR with external standards. Target genes exhibiting mRNA expression correlating with Tcf-4 were further investigated. In silico promoter screens for potential Tcf-4 binding sites (WWCAAWG) and gel shift assays for in vitro confirmation were performed. Total Protein from ileal biopsies of controls and ileal CD patients was extracted and analyzed via Western Blot.

Results. In addition to the known decrease of Tcf-4, we found reduced expression and protein of the Wnt pathway transcription factors Tcf-1 in ileal (p=0,0242), but not colonic CD. The mRNA levels of Tcf-1 correlated with Tcf4 (rs=-0,5206; p<0,0001) as well as with HD5 and 6 (p<0.0001). Among the 4 potential Tcf-4 binding sites identified in the Tcf1 promotor, a region between -2492 and -2485bp upstream of the transcription start, exhibited the strongest capability for Tcf4- binding. In contrast to α-defensins, most other Wnt pathway and Tcf-4 target genes (26 total) were either unchanged (22) or increased (MMP7: p= 0,0759; Claudin 1: p=0.032;PGLYP-1: p=0,0446).

Conclusion. Wnt signaling regulated antimicrobial defense is disturbed in ileal CD. Tcf-4, its target Tcf-1, as well as HD5 and HD6 exhibit decreased gene expression. However, other major pathway targets are unchanged, suggesting a selective function of Tcf-4 in intestinal innate immunity. Since Tcf-1 is an important Wnt pathway transducer in T- cells, its down regulation in ileal CD could also constitute a link to adaptive immunity.

DMBT1 CONFERS MUCOSAL PROTECTION *IN VIVO* AND A DELETION VARIANT IS ASSOCIATED WITH CROHN`S DISEASE

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Impaired mucosal defense plays an important role in the pathogenesis of Crohn`s disease (CD), one of the major subtypes of inflammatory bowel disease (IBD). Deleted in Malignant Brain Tumors 1 (DMBT1) is a secreted scavenger receptor cysteine-rich protein with predominant expression in the intestine and has been proposed to exert possible functions in regenerative processes and pathogen defense. Here, we aimed at analyzing the role of DMBT1 in IBD. We studied DMBT1 expression in IBD and normal tissues by quantitative RT-PCR, immunohistochemistry and mRNA *in situ* hybridization. Genetic polymorphisms within *DMBT1* were analyzed in an Italian IBD case-control sample. *Dmbt1*$^{-/-}$ mice were generated, characterized, and analyzed with regard to their susceptibility to dextran sulfate sodium-induced colitis. The *DMBT1* levels correlate with disease activity in inflamed IBD tissues. A highly significant fraction of the IBD patients displayed upregulation of DMBT1 specifically in the intestinal epithelial surface cells and Paneth cells. The deletion allele of *DMBT1* with a reduced number of scavenger receptor cysteine-rich domain coding exons is associated with an increased risk for Crohn`s disease (P = 0.00056; OR: 1.75), but not for ulcerative colitis. *Dmbt1*$^{-/-}$ mice display enhanced susceptibility to dextran sulfate sodium-induced colitis and elevated Tnf, Il6, and Nod2 expression levels during inflammation. We conclude that DMBT1 may play a role in intestinal mucosal protection and prevention of inflammation. An impaired DMBT1 function may contribute to the pathogenesis of Crohn`s disease.

PARAEPITHELIAL PASSAGE OF ADHERENT LUMINAL BACTERIA VIA MODIFIED CELL-CELL-CONTACTS IN CROHN´S DISEASE

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Scientific Interests:
Intestinal expression of pattern recognition receptors and their role in graft-versus-host disease after allogeneic bone marrow transplantation

Among polygenetic and environmental factors the intestinal microflora seems to play an essential role in the pathogenesis of Crohn's Disease (CD). Autoprotective features such as the apical mucus or epithelial cell-cell-contacts assure the integrity of the intestinal barrier. To date it is unclear if endogenous bacteria are directly or indirectly involved in the initiation or perpetuation of CD. Inflammation, as seen in CA, could be the result of active translocation of luminal bacteria into the *Lamina propria* via a leaky mucosal barrier. Using 16S-rRNA-FISH-technology we demonstrated a distinct/profound bacterial colonisation of the intestinal mucus of CD-patients while in controls no bacteria were detected. Translocation of adhesive bacteria (or their products) in CD was proven by accumulation of endotoxin in the intestinal tissue, which was even more pronounced in patients carrying NOD2/CARD15 mutations. Control patients showed no intramucosal endotoxin staining. Electron microscopy revealed no morphologic changes of cell-cell-contacts in the intestinal tissue of CD patients. However, tight junction proteins controlling pore formation displayed a higher expression in CD tissue, whereas sealing adherens junction proteins were diminished. These data strongly suggest that dysregulation of autoprotective barrier functions play a role in CD pathogenesis. Mucus colonisation allows luminal bacteria and their products to get in contact with the apical surface of the epithelium and to cross the intestinal barrier via altered junctions. This unphysiological influx of antigens might contribute to the chronification of intestinal inflammation.

INTERLEUKIN 10 INHIBITS ENDOPLASMATIC RETICULUM AND MITOCHONDRIAL UNFOLDED PROTEIN STRESS RESPONSES IN THE INTESTINAL EPITHELIUM: IMPACT ON CHRONIC INFLAMMATION

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The initiation of endoplasmic reticulum (ER)-mediated stress responses in intestinal epithelial cells (IEC) may contribute to the pathogenesis of chronic intestinal inflammation. The aim of the study was to characterize ER stress response mechanisms in IL-10 deficient (IL-10-/-) mice developing experimental colitis.

Primary IEC were isolated from IL-10-/- and wild type mice after the mono/dualassociation with colitogenic *Escherichia coli* and *Enterococcus faecalis*. Western Blot analysis and mRNA expression in primary IEC of inflamed IL10-/- mice revealed increased expression levels of grp78 under conditions of experimental colitis. Interestingly, the induction of ER stress response mechanisms was associated with decreased expression levels of the mitochondrial creatine kinase (MtCK).

Consistent with the results of primary IEC from inflamed IL-10-/-mice, *in vitro* experiments with IL-10 receptor reconstituted Mode K (IL-10R) stimulated with ER stress inducer tunicamycin revealed inhibitory effects of IL-10 on the protein expression level of grp78. Additionally IL-10 inhibits the ER stress-induced loss of MtCK which has an important role in structure of mitochondria and and a loss of MtCK may contribute to decreased stability of the mitochondrium.

Induction of ER stress in IL-10R by tunicamycin leads to transcriptional upregulation of nuclear genes encoding mitochondrial stress proteins (grp75 and matrix protease ClpP) envolved in mitochondrial unfolded protein response (mtUPR). Immunoprecipitation of grp75 showed that an upregulation of ClpP is associated with downregulation of MtCK implicating a link between mtUPR and ER stress response mechanisms. Additional IL-10 treatment rescued the loss of MtCK suggesting an important role of IL-10 on energy homeostasis under the conditions of mtUPR in the intestinal epithelium.

HYDROGEN SULFIDE AS A NOVEL NEUROMODULATOR

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Hydrogen sulfide (H_2S) has been suggested as a novel gasomediator in the brain and vascular system. Our results suggest H_2S as a neuromodulator in the gut enteric nervous system. We used immunohistochemistry to detect H_2S -producing enzymes cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS) in enteric neurons of guinea-pig and human. The exogenous H_2S donor NaHS (0.2-2.5mM) concentration-dependently increased ion secretion in human and guinea-pig submucosa/mucosa preparations, but not in the colonic epithelial cell line T84. The pro-secretory effect was due to activation of cAMP and Ca dependent chloride channels. Although the pro-secretory effect of NaHS in intact tissue was fully abolished by the neurotoxin TTX, we did not observe changes in intracellular calcium of isolated enteric neurons. We did, however, observe an increased spike discharge in enteric neurons in intact tissue preparations. The explanation for this result is that NaHS first activates TRPV1 expressing extrinsic visceral nerves which then release mediators that activate enteric neurons. This is suggested by the powerful inhibitory action of the TRPV1 antagonists Capsazepine and AMG 9810. Extrinsic visceral nerves release Substance P upon activation. Consequently, the NaHS induced secretion was inhibited by the NK1- antagonist SR 140333 and the NK3 antagonist SR142801. We conclude that H_2S is a novel neuromediator in the gut. It is synthesized by enteric neurons and feeds back on TRPV1 expressing extrinsic visceral nerves. H_2S is thereby an important signalling molecule for communication between intrinsic and extrinsic nerves, the latter being mostly nociceptive fibers. Excessive H_2S concentations released from the microflora, blood cells or enteric neurons my lead to hyperactivity in the enteric nervous system. According to our concept this may lead to hypersecretion and visceral hypersensitivity.

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