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Inflammatory Bowel Diseases: Microbiota versus the Barrier

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Abstracts
Poster Abstracts
Falk Symposium 188

INFLAMMATORY BOWEL DISEASES:
MICROBIOTA VERSUS THE BARRIER

Stuttgart (Germany)
June 7 – 8, 2013

Scientific Organization:
E.F. Stange, Stuttgart (Germany)
A. Dignass, Frankfurt (Germany)
K. Fellermann, Lübeck (Germany)
K. Herrlinger, Hamburg (Germany)
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Session I

The gut microbiota and the mucosa in IBD
Keynote Lecture

History and philosophy of IBD

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Since the discovery of two chronic inflammatory intestinal disorders many concepts on the diseases have evolved that tried to explain their pathophysiology and had consequences for the search for new therapeutic approaches. I may not be necessary to review those concepts as to learn something about the disease, but it may help to value present concepts and their role in understanding IBD.

Many good scientists still believe in Sir Carl Popper’s theory of science. He postulated that with science we achieve a more and more complete picture of the real world, an asymptotic approximation to the truth. Popper postulated that this happens via falsification of hypotheses. As long as we exclude more and more false hypotheses by experimental falsification we learn more and more about the “truth” or “reality”. The concept has several problems: First there might be an infinite number of hypotheses and we falsify until the cows come home without getting an idea about how nature works. Second it remains unclear how we generate good hypotheses and how they can be related to the “truth” at all.

So there is just one possible conclusion: Pooper was falsified (indicating another problem: What remains if everything is ‘falsified’?).

Science in general and subsequently IBD research must be seen as a historical succession of pre-ideas, concepts, scientific trends and sometimes scientific “revolutions” as indicated by Thomas S. Kuhn.

Which concepts have played a lead role in IBD research and which concepts do we face now?

First of all our modern concept of disease has evolved in the 19th century. Therefore, a disease such as Crohn’s disease could not really be discovered before. However, certainly there were historic persons that had an illness and symptoms that may retrospectively be interpreted as Crohn’s or colitis such as Alfred the Great, King of the Anglo-Saxons or King Luis XIII from France. Nevertheless medicine at their time was focussed on separate symptoms and usually not able to integrate a complex of symptoms to a concept of a specific disease. Such a concept was there in the early 20th century allowing Burrill B. Crohn to describe a number of patients with similar disease features a suffering from ONE DISEASE that eventually would be named after him. At that time the concept of an infectious agent usually causing a disease was very modern and Crohn himself believed that the disease he described was an infectious disease. He favored mycobacteria to be responsible.

Despite the lack of a proof for this concepts and many arguments against it (“falsifications” in Popper’s sense) the concept of an infectious agent causing Crohn’s disease is still present and supported by a number of researchers (this by the way is an argument against Thomas S. Kuhn: “revolutions” frequently do not cause scientific concepts to completely disappear). Other concepts have evolved in the past decades with more or less success. The concept of a failure in adaptive immunity (“T-cell
disease”) was followed by a concept explaining the diseases with a failure in “innate immunity”. This is now followed by the concept of a disturbance in the “microbiome”. In parallel we had the “genetic concept” for IBD pathophysiology. Some aspects of these concepts always can be combined, others are exclusive.

The success of GWAS studies and genotyping has mislead some colleagues to postulate that in a few years we will have many “Crohn’s diseases” depending on the respective pathophysiology. However, this is not how a concept of a disease can evolve. An example might be arteriosclerosis. The pathophysiological events at the intima of arteries are well understood, a number of specific pathways leading to arteriosclerosis have been identified, risk genes have been discovered and risk factors analyzed. Nevertheless there are not many different “arteriosclerosis diseases” as the disease concept concentrates on the consequences of those processes and the clinical presentation. The clinical presentation defines the disease which is also true for Crohn’s and colitis.

There are only limited ways for complex systems as our body to react or the other way around: we have only limited resources to comprise and differentiate reactions of our body. Thus new scientific techniques such as arrays, sequencing, microbiome analysis and perhaps other upcoming techniques will modify our concept of IBD. A certain core concept however, will remain relatively constant (as long as we do not change the concept of “disease” in general). At the end we need to cross the borders of our “conceptual communities” within the “IBD community” to achieve the most benefit for our patients.

Considering the “history and philosophy of IBD” may help a little for this process and prevent us from taking our own research and concepts too serious.
An overview of gut microbiota: Human gut microbiota and its dysfunctions

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A complete understanding of the human biology will only be fully assessed by combining the analysis of the host and its surrounding environment. The human gastrointestinal tract hosts more than 100 trillion bacteria and archaea, which together make up the gut microbiota. Even though the amount of bacteria in the human gut outnumbers human cells by a factor of 10, some finely tuned mechanisms allow these microorganisms to colonize and survive within the host in a commensalism relationship. The human gut microbiota can be considered an organ within an organ that co-evolved with humans to achieve a symbiotic relationship leading to physiological homeostasis. The human host provides a nutrient-rich environment and the microbiota provides indispensable functions that humans cannot exert themselves.

Chaotic in the early stages of human life, the assembly of the human gut microbiota remains globally stable over time in healthy conditions in the absence of perturbation. The average total number of bacterial species was estimated to be close to 1000 per individual. The restricted number of phyla in comparison to other ecosystems has suggested a tight co-evolutionary history between the host and its microbiota. Remarkably, shifts in the bacterial makeup of the human gut microbiota have been associated with digestive tract dysfunctions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and obesity. More than 10 years ago, the concept of dysbiosis or unbalanced composition of the intestinal microbiota, was introduced in the IBD research field.

Further insights into the human gut ecosystem are needed to comprehend the exact role of microbiota in health and disease. Because most of the bacteria inhabiting the gut are uncultivable, their functions cannot be inferred from composition data. Knowing which microbes are there is not sufficient. Meta-omics has been developed to answer essential questions such as “What is the genetic potential of the non-cultured bacterial fraction of the gut microbiota?” and “What are these microbes really doing?”
Culture-dependent and -independent analyses of mucosal-associated bacteria revealed that patients with IBD have less complex profiles of commensal bacteria and higher numbers of mucosa-associated bacteria than healthy individuals. The microbiota is remodelled during the active phase of CD, with the equilibrium leaning toward dominant phylogenetic groups, perhaps as a consequence of inflammation. Patients with IBD, compared to healthy controls, have fewer bacteria with anti-inflammatory properties and/or more bacteria with pro-inflammatory properties. Several metagenomic-based studies reported that members of the phyla Bacteroidetes and Firmicutes were reduced in patients with CD or UC. Reduced numbers of Bacteroides fragilis might contribute to inflammation because this prominent human symbiont has protective effects – it protects mice from colitis induction by Helicobacter hepaticus, a murine commensal bacterium with pathogenic properties. Among Firmicutes, Faecalibacterium prausnitzii has anti-inflammatory properties; its numbers are reduced in patients with CD and associated with risk of post-resection recurrence of ileal CD.

In contrast, a greater relative abundance in Enterobacteriaceae, mostly Escherichia coli, was observed in CD patients, more notably on mucosa-associated microbiota than in fecal samples. Several independent studies have reported increased numbers of mucosa-associated E. coli with invasive properties or the presence of intramucosal E. coli in IBD patients. These pathogenic E. coli, compared to commensal E. coli, have acquired specific virulence factors that increase their ability to adapt to new niches and allow them to cause disease. Microbial analyses of CD specimens indicate also that the ratio of F. prausnitzii:E. coli could be used to evaluate the level of the dysbiosis in IBD patients and identify those at high risk for recurrence of CD. In addition, fluorescent in situ hybridization analyses showed the presence of bacteria that penetrate the mucus layer in 30% of mucosal biopsies from patients with IBD, compared with 3% of mucosal biopsies from healthy controls indicating that the microbiota might have closer contact with the mucosa of IBD patients. This might result from the increased numbers of some mucolytic bacteria, such as Ruminococcus gnavus and Ruminococcus torques, observed in macroscopically and histologically normal colonic epithelium from patients with colonic CD and UC.

The dysbiosis observed in IBD patients, with subsequent disruption of the intestinal microbiota community, might arise from colonization by an enteric pathogen, from host-mediated inflammatory responses, or from a combination of these. Pathogens could subvert the inflammatory response and then take advantage of inflammation to breach the barrier effect imposed by the resident microbiota and the intestinal mucosa itself. In addition with the identification, in CD patients with ileal involvement of the disease, of mutations in genes involved in the autophagy process (NOD2, ATG16L1, IRGM), any enteric pathogens with intracellular life-style could be involved.
Diet and the intestinal microbiota in IBD

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The increasing incidence of inflammatory bowel diseases (IBD) is considered to be the consequence of environmental and individual risk factors. Despite the fact that the intake of high fat diets and meat was associated with increased risk for Crohn’s disease and ulcerative colitis, the mechanistic role of diet as a key environmental factor in the pathogenesis of chronic intestinal inflammation is not yet established. A major focus of research into disease mechanisms underlying chronic inflammation is the gut microbial ecosystem and its interaction with the intestinal mucosa. However, the composition and activity of the intestinal microbiota is largely affected by dietary factors and, both intermediates might play an interrelated role in orchestrating disease risk and activity. We studied the role maternal inflammation, dietary factors including iron, high-fat and semi-synthetic diets in modulating disease activity in IBD-related animal models and IBD patients.
The host and the flora

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To prevent bacterial overgrowth, colonization of the epithelium and subsequent translocation, the gastrointestinal tract maintains an effective mucosal barrier. Besides mucus the most important components of this protective system are epithelial antimicrobial peptides such as defensins, the cathelicidin LL-37, lysozyme, phospholipase A, and proteins with additional antimicrobial properties such as ubiquicidin, ribosomal proteins or histones. Commensal species may tolerate intestinal antimicrobial peptides, for example Bacteroides ssp. or Parabacteroides ssp. as major species in the human colon were highly resistant to the constitutive defensin HBD1 and only susceptible to the inducible defensin HBD3. Reduction of disulfide bonds is an important mechanism activating HBD1. As several studies show, alterations in the expression of antimicrobial peptides directly influence the composition of the intestinal flora. Correspondingly increased production of defensins or inhibition of the processing of mouse defensins to their active form led to a quantitative shift of luminal and mucosal bacterial species.

On the other hand microorganisms also modulate the synthesis of host defensins by induction or inhibition of specific peptides. Lactobacilli, the probiotic strain E. coli Nissle and Salmonella enteritica stimulate HBD2 expression, whereas Shigella flexneri downregulates the synthesis of HBD1, HBD3 and LL-37.

Thus, the proper balance between the luminal flora and the mucosa is a permanently dynamic, sensible and host-specific relationship.
Session II

The gut barrier in IBD:
The first line of defence
Intestinal stem cells

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The intestine has become a prime model system to study stem cell biology. Intestinal stem cells can be identified based on the expression of a unique marker gene, namely Lgr5. Transgenic mouse models expressing green fluorescent protein (gfp) in intestinal stem cells have allowed their visualization, isolation, molecular characterization and use in generating organoids: small mini-guts that contain all cell types of the intestine. Detailing the behavior of intestinal stem cells has also led to new insights concerning the mechanism of self renewal versus differentiation. Genes and pathways directing daughter cells of stem cells towards the differentiated lineages of the intestine are getting better defined. Of all differentiated cells, Paneth cells play a distinguished role: they emerged from pure bystanders to the guardians of the stem cell. But Paneth cells also have a “dark” side: they also act as supporters of intestinal cancer stem cells. Taken together, a detailed molecular picture emerges that describes the mechanisms of intestinal homeostatic self-renewal and outlines new therapeutic avenues.
Nets and harpoons: Innate immune functions of α-defensins in the small intestine

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The intestinal mucosa interfaces with a complex, dense community of microorganisms, including hundreds of species of resident microbiota and many transient microbes entering from food and water borne sources. In the small intestine, Paneth cells (specialized secretory epithelial cells) produce abundant quantities of α-defensins and several other antibiotic peptides [1, 2]. Human Paneth cells make two α-defensins: HD5 and HD6. Data from in vivo models indicate that Paneth cell α-defensins play a pivotal role in defense from food and water borne pathogens in the intestine [3, 4]. The mechanism by which these two α-defensins protect from enteric pathogens is quite distinct. HD5 is a potent antimicrobial that kills target microbes by membrane disruption (harpoons) [3], whereas HD6 is newly discovered to self-assemble to form fibrils and nanonets that surround and entangle bacteria (nets) [4]. Recent data suggest that HD5 also serves to help shape the composition of the colonizing microbiota [5]. Studies in humans suggest that reduced expression of HD5 and HD6 is a fundamental feature of ileal Crohn’s disease [6, 7]. Mechanistically, the link between reduced Paneth cell α-defensin expression and ileal Crohn’s disease pathogenesis may be a result of the weakened mucosal antimicrobial defense and/or alterations in the composition of commensal microbiota.

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Mucus and the goblet cells

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The discovery that an inner colon mucus layer separates the commensal bacteria from the epithelial cells has opened a new research arena where the formation and properties of this dynamically controlled system is directly linked to colon inflammation. The outer mucus colon mucus layer is on the other hand the habitat for the commensal bacteria and the mucin an important food source.

We have studied a number of mouse models for colitis (Muc2\textsuperscript{-/-}, Core 1 O-glycans\textsuperscript{-/-}, Tlr5\textsuperscript{-/-}, IL10\textsuperscript{-/-} and Slc9a3 [Nhe3]\textsuperscript{-/-}, together with dextran sulfate, DSS). All these murine colitis models revealed bacteria in contact with the epithelium. Additional analysis of IL10\textsuperscript{-/-} mice with low inflammation revealed a thicker mucus layer than WT, but the properties were different as the inner mucus layer was penetrated both by bacteria \textit{in vivo} and by fluorescent beads the size of bacteria \textit{ex vivo}. Clear separation between bacteria or fluorescent beads and the epithelium mediated by the inner mucus layer was also evident in normal human sigmoid colon biopsies, but in contrast, mucus on colon biopsies of ulcerative colitis (UC) patients with acute inflammation had highly penetrable mucus. Most UC patients in remission had an impenetrable mucus layer, but some had penetrable mucus.

Normal human sigmoid colon thus has an inner mucus layer impenetrable to bacteria. However, the inner colon mucus in animal models with spontaneous colitis and in UC patients with active disease is penetrable to bacteria. Thus the inner colon mucus layer is important for protecting the epithelium and its properties can be modulated by for example the immune system. This suggests a novel and unifying model of UC pathophysiology.

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Defective antibacterial barrier in IBD

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The intestinal barrier is a delicate structure composed of a single layer of epithelial cells, the mucus, commensal bacteria, immune cells, and antibodies. Furthermore, a wealth of antimicrobial peptides (AMPs) can be found in the mucus and defend the mucosa. Different lines of investigations now point to a prominent pathophysiological role of defensins, an important family of AMPs, in the pathogenesis of inflammatory bowel disease and, particularly, in small intestinal Crohn’s disease. This talk will briefly summarize the different antimicrobial peptides of the intestinal mucosa and describe their function, their expression pattern along the gastrointestinal tract, and their spatial relationship to the mucus layer. Also, alterations found in inflammatory bowel disease will be presented. Small intestinal Crohn's disease (CD) is closely linked to defects in Paneth cells (specialized secretory epithelial cells at the bottom crypts) which secrete α-defensin human defensin (HD)-5 in huge quantities in healthy individuals. Decreased expression of these antimicrobial peptides is found in ileal CD, and single nucleotide polymorphisms with the highest linkage to CD affect genes involved in Paneth cell biology and defensin secretion. Additionally, antimicrobial peptides have a role in ulcerative colitis, where the depleted mucus layer cannot fulfill its crucial function of binding defensins and other AMPs to their proper site of action. Inflammatory bowel disease arises when the mucosal barrier is compromised in its defense against challenges from the intestinal microbiota. In ileal CD, a strong association can be found between diminished expression or defective function of defensins and the advent of intestinal inflammation. In summary, inflammatory bowel diseases are characterized by compromised antimicrobial barrier function which is mediated by a variety of complex mechanisms.
Permeability and tight junctions in IBD

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Increased intestinal permeability is a feature of intestinal inflammation and has been implicated as a pathogenic factor in inflammatory bowel disease (IBD). This has been further confirmed by the development of genetic studies, where several of the 163 identified risk loci for IBD are related to barrier function.

The epithelial phenotype in active IBD is very similar in Crohn’s disease and ulcerative colitis. It is characterized by increased secretion of chloride and water, increased epithelial permeability, and increased apoptosis of epithelial cells. Paracellular permeability alterations in IBD comprise increased myosin light chain phosphorylation, a reduced number of tight junction strands and distorted expression and distribution of important tight junction proteins, such as claudins, JAM-A and ZO-1. In early stages the intestinal barrier impairment can also result from transcellular transport of antigens via endocytotic uptake into early endosomes. In Crohn’s disease, the permeability changes of both the trans- and paracellular routes are mainly driven by TNF. In ulcerative colitis, on the other hand, increased paracellular permeability is dependent on IL-13, whereas the increased transcellular antigen transport may be induced by mast cell activation following release of corticotropin-releasing factor from eosinophils. The presentation will give an overview of the key players of the mucosal barrier, review the current literature on mechanisms of mucosal barrier dysfunction, and suggest potential therapeutic strategies to restore barrier function in IBD.
Session III

The gut barrier in IBD:
The second line of defence
State-of-the-Art Lecture

An overview of innate and adaptive cellular immunity in IBD

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Inflammatory bowel diseases (Crohn’s disease, ulcerative colitis) are chronic inflammatory disorders of the gastrointestinal tract. Studies on disease pathogenesis have suggested that IBD develop by uncontrolled activation of the mucosal immune system in a genetically susceptible host. This activation appears to be triggered by antigens from the commensal microflora.

Recent studies have unequivocally demonstrated that both innate and adaptive cellular immunity are altered in IBD. This includes changes in antimicrobial host defense and immune responses by intestinal epithelial cells, Paneth cells, granulocytes, macrophages and dendritic cells as well as marked alterations of lymphocyte responses including B and T cell responses. These changes are not only relevant for the pathogenesis but also for the therapy of IBD.

This presentation will give a brief overview about the observed changes in innate and adaptive immune responses in IBD. The implications of recent findings on the design on novel therapeutic approaches for IBD as well as for individualized therapy in IBD will be discussed.
What is wrong with granulocytes in IBD?

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The hallmark of the pathologic lesion in Crohn’s disease is a chronic inflammation characterised by granulomata which generally signify the presence of undigested or indigestible material in the tissues. The primary function of granulocytes is to phagocytose, kill and digest bacteria and fungi that penetrate into the tissues. They also remove exogenous and endogenous organic material and debris. Faecal contents enter the wall of the bowel following mucosal damage by insults such as infection with viruses, bacteria or other organisms and trauma. The removal of this material from the tissues requires efficient neutrophil function in the absence of which the foreign material is walled off by macrophages to form granulomata. The retained material results in fibrosis and stricture formation and fistulation. A very high proportion of patients with primary immunodeficiencies affecting neutrophils, like CGD [1], develop Crohn’s like lesions of the colon and perianal fistulation. In most patients with Crohn’s the neutrophils are themselves normal, but are not attracted to sites of inflammation in the bowel [3] or at other sites [2, 4], as a result of which bacteria are cleared very slowly. The primary cause of this delayed accumulation of neutrophils appears to be an impairment of the secretion of pro-inflammatory cytokines from macrophages because of abnormal trafficking of cytokine bearing vesicles [4]. MDP dependent NOD2 signalling can over-ride this defective chemoattraction except in individuals with Crohn’s related mutations in this molecule [3].

References:

Local control of dendritic cell and macrophage heterogeneity in intestinal homeostasis and inflammation

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Dendritic cells (DC) and macrophages play complementary roles in maintaining immune homeostasis in the intestine, by inducing the differentiation of gut homing regulatory T cells and acting as disposal units for bacteria and damaged cells respectively [1, 2]. However defining the exact function of these cell types has been difficult due to the fact that they express overlapping markers such as CD11c and class II MHC and they also behave very differently from their counterparts in other tissues. As a result, there is considerable confusion in the field about the functions, ontogeny and roles of intestinal mononuclear phagocytes (MP). Clarifying these issues would be important for understanding the pathogenesis of disorders such as coeliac disease and Crohn's disease, for designing targeted therapies and for vaccine development.

We have established multiparameter flow cytometry techniques which allow us to distinguish clearly, several subsets of bona fide DC and macrophages in mouse intestine. In the healthy colon, most resident macrophages in resting mouse colon express very high levels of CX3CR1, are avidly phagocytic and MHCIIhi, but are resistant to TLR stimulation, produce IL10 constitutively, and express CD163 and CD206 [3]. Unlike resident macrophages in other tissues, these are derived from continuous replenishment by blood Ly6Chi monocytes which differentiate locally and acquire the unique gut phenotype within a few days after their arrival [3, 4]. Similar selective processes control the development of DC in the intestine, where common DC precursors (pre-cDC) give rise to four distinct subsets of DC which can be identified based on the expression of CD11b and CD103 and which do not appear in other tissues. Each of these subsets has distinct functions tailored to particular needs of the intestinal environment and together these results indicate that the biology of myeloid cells in the intestine is determined by unique signals in the mucosa itself that control precursor differentiation. In the case of monocytes-macrophages, these local processes are arrested during experimental colitis and in patients with Crohn's disease, resulting in accumulation of TLR-responsive pro-inflammatory macrophages and tissue pathology [3]. It seems likely that similar dysregulation of DC development may also occur in inflammation and understanding the various processes involved in these events could offer routes for therapeutic intervention in IBD.

References:


**What is wrong with T and B lymphocytes?**

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Both innate immunity and adaptive immunity play an important role in the pathogenesis of inflammatory bowel diseases (IBD). Whereas in the past mainly various cytokines have been targeted especially by neutralizing antibodies as treatment option in human IBD, recent studies have also used “cellular targeted strategies”.

Especially T cells but also B cells are crucially involved in these diseases. Adaptive immune processes play a major role in both human IBD and histology is characterized by both infiltrating T and B cells. There is indeed strong evidence implicating T cells and T-cell migration to the gut in initiating and perpetuating intestinal inflammation and tissue destruction. Major progress has been made in recent years in our understanding of the mechanisms leading to the generation of “gut-homing” effector T-cell populations. Several studies have demonstrated that the gut-homing receptors CCR9 and α4β7 are selectively induced on T cells during their priming in intestinal inflamed sites. The increased numbers of CD4⁺ T cells in human IBD may be explained by enhanced influx/activation and decreased apoptosis of mucosal T cells. Whereas targeting activated CD4⁺ T cells by antibody strategies or neutralization of key T-cell cytokines such as IL-2 or IFNγ has not been effective in human IBD, the recently chosen approach blocking influx of activated leukocytes e.g. T cells into the inflamed tissue by specific antibodies such as vedolizumab seems highly effective. Recently it could also been demonstrated that administration of antigen-specific regulatory T cells to patients with refractory Crohn’s disease was not only well tolerated but showed promising results.

SAMP1/YitFc mice develop transmural inflammation mainly in the terminal ileum with similarities to Crohn’s disease. In this model, B cells are massively expanded and have been shown to block regulatory T cells thereby exacerbating disease. Therefore, it would have been expected that targeting B cells in human disease could be of benefit. B-cell depletion has so far only been studied in a controlled manner in ulcerative colitis. A recently reported study failed to show any clinical benefit for rituximab therapy in ulcerative colitis. Furthermore, several case reports described de novo ulcerative colitis after rituximab therapy.

Therefore, although targeting “inflammatory“ T and B cells directly has not been successful in previous studies, treatments targeting migration of activated T cells or using regulatory T cells are very promising.
Session IV

Diagnostics and prognostics in IBD
Antimicrobial antibodies

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IBD diagnosis may be straightforward, using clinical, endoscopic, histologic and imaging studies. However, in ~10–15% of patients with disease limited to the colon, a firm diagnosis of ulcerative colitis (UC) or Crohn’s disease (CD) cannot be established; a condition designated inflammatory bowel disease (IBD)-undetermined (IBD-U). In this situation, as well as in the pediatric population where IBD-U is diagnosed more frequently additional tests—such as antimicrobial antibodies, may be needed for disease diagnosis and differentiation.

Antimicrobial antibodies are characteristic of CD, are directed against microbial antigens—usually proteoglycans, and their existence, especially in high titers and when there is response against more than one antigen is usually associated with a more aggressive disease phenotype. The exact link between innate and adaptive immune responses that results in the formation of these antibodies is still unclear. In addition to their potential contribution to differentiation between CD and UC, antimicrobial antibodies may enable improved disease stratification into high risk groups, thus potentially contributing to disease management. For that reason, it seems plausible to assess antimicrobial antibodies at diagnosis, serving diagnostic and prognostic needs. We will review the major serologic markers including ASCA (anti-Saccharomyces cerevisiae antibodies); OmpC (anti-Escherichia coli outer membrane porin C); Anti-I2 (anti-Pseudomonas fluorescens sequence I2); Anti-CBir1 Flagellin (anti-flagellin of commensal bacteria); the novel anti-glycan antibodies: ALCA (anti-laminaribioside carbohydrate antibodies); ACCA (anti-chitobioside carbohydrate antibodies); AMCA (anti-mannobioside carbohydrate antibodies); Anti-L (anti-laminarin) and Anti-C (anti-chitin), Anti-GP2 (anti-glycoprotein 2), as well as ANCA (anti-neutrophil cytoplasmic antibodies), more characteristic of UC; The presumed antigens will be described, as well as the association of the various markers with different disease phenotypes. The occurrence of antibodies against microorganisms in IBD, specifically CD patients, suggests several pathogenetic mechanisms explaining this phenomenon. These will be described and discussed.
Diagnostics and prognostics in IBD: Fecal neutrophil-derived biomarkers calprotectin and lactoferrin

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Crohn’s disease (CD), ulcerative colitis (UC), and colitis unclassified, collectively defined as inflammatory bowel disease (IBD), are the consequence of chronic inflammatory reactions in the gastrointestinal tissue. Endoscopy with biopsies is the mainstay in the diagnosis of this inflammation and is also important in the assessment of disease activity and monitoring of treatment. Furthermore, mucosal healing is increasingly becoming a therapeutic target for treatment of IBD and the golden standard of assessing it is endoscopy. However, due to the costs, invasiveness, and to limited endoscopic capacity, the need is strong for reliable surrogate markers of intestinal inflammation. Bowel contents, being in close contact with intestinal mucosa, can take up molecules that are measurable from stool samples and thus can serve as markers of inflammation. The fecal neutrophil-derived biomarkers, especially calprotectin and lactoferrin have several features of an ideal test for detecting intestinal inflammation: They are non-invasive, simple, and low in cost. The utility of these biomarkers in distinguishing IBD from non-inflammatory conditions such as irritable bowel syndrome is well documented. They correlate closely with endoscopic activity both in CD and UC. They allow serial monitoring of disease activity and of treatment success, and can even serve in predicting clinical relapse in asymptomatic patients or sustained remission after induction with TNF-α-blocking agents. In this review an overview will be given to the role of fecal neutrophil-derived biomarkers calprotectin and lactoferrin in diagnostics and prognostics of IBD.
Imaging update

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Diagnostic imaging approaches play an important role in the diagnosis and management of patients with inflammatory bowel diseases (IBD). The diagnostic approach should be guided by considerations of diagnostic accuracy, concerns about patient exposure to ionizing radiation and tolerance of the endoscopic and/or imaging technique. In Europe abdominal ultrasound is considered as the primary imaging approach for the evaluation of the abdomen, however, this technique is not employed in the United States. For the evaluation of the small bowel MR- and CT-enterography can be considered as gold standard, but in selected cases capsule endoscopy or balloon endoscopy may provide meaningful additional information for the management of IBD patients. Currently new experimental imaging approaches in IBD include PET-CT, newer MR-imaging modalities to evaluate the degree of intestinal fibrosis/inflammation and capsule endoscopy of the colon.

References:

Endoscopy as a prognostic marker in IBD

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In patients suffering from duodenal ulcers or reflux esophagitis, the severity of the lesions and the persistence of mucosal lesions help predicting a poorer prognosis and adapt treatment strategy. In my lecture, I will be critically reviewing the data which suggest that mucosal healing has a prognostic value also in IBD.

In the nineties, the general opinion, based on a few studies was that assessing endoscopic lesions was not critical for the management of IBD. Arguments were that endoscopic evidence of mucosal healing was not necessarily associated with histological healing, and that using steroids for longer periods in patients with Crohn’s disease who did not reach endoscopic healing did not lead to a better prognosis. The more recent therapeutic strategies using less steroids and more immunosuppressants or biologics lead to a higher percentage of mucosal healing, and there is growing evidence that reaching healing of lesions is of good prognostic value both in ulcerative colitis and Crohn’s disease. There is a lower risk of hospitalisation and of surgery in patients with mucosal healing than in those without healing. There is also a lower risk of relapse after stopping treatments in patients with mucosal healing than in those with persistent lesions. The risk of cancer (on a long term basis) seems also be lower in patients with controlled inflammatory lesions than in controls with persistent lesions.

It is thus now important to check lesions in the treatment strategy but many questions of paramount importance are still open: when to check (in order to avoid excessive indications of endoscopies)? what is the treatment goal? (healing of any lesion? healing of ulcers only? histological healing?) and what should be done when the target is not reached (change the treatment?).
Session V

Differences between treatment guidelines in IBD
In 2006, ECCO published its first set of guidelines ever. These guidelines covered the diagnosis and current management of Crohn’s disease and were followed by guidelines on the diagnosis and current management of ulcerative colitis in 2008. The guidelines were initially published in GUT, before they were moved to ECCO’s own journal JCC (Journal of Crohn’s & Colitis). These guidelines soon became standard references for the management of IBD in Europe and in other parts of the world. The articles on current management of ulcerative colitis and Crohn’s disease are among of the top-cited papers published in GUT and JCC illustrating very well how the ECCO guidelines have impacted on the community of physicians caring for patients with IBD.

In the meantime, ECCO has developed and published additional guidelines related to various problems in IBD care, e.g., management of opportunistic infections in IBD, pregnancy and reproduction in IBD, imaging, endoscopy and histopathology in IBD and management of pediatric UC and CD.

Since the evidence and current practice that are the basis for the guidelines change over time, ECCO opted to revise its guidelines on a regular basis (usually every 4–5 years) and several guidelines have been updated and revised in the meantime. The development of ECCO guidelines has been further improved and standardized over the past years. The importance of guidelines for ECCO is highlighted by the foundation of a Committee (GuiCom) just responsible for all the issues around ECCO guidelines. Under the supervision of GuiCom standard operating procedures (SOPs) for ECCO guidelines have been developed to facilitate the selection and preparation of guideline projects by ECCO, to increase transparency of the entire process leading to novel guidelines or updates of established guidelines and to facilitate and standardize the dissemination and publication of ECCO guidelines. ECCO guideline proposals may be submitted by any individual ECCO member. Every proposal will be reviewed by GuiCom and ultimately approved or rejected by ECCO’s governing board. The selection of guideline coordinators and participants involves open calls to all ECCO members via ECCO e-newsletters announcing the guideline project. The selection of working party members (ECCO members and external experts) and working party leaders is a combined responsibility of GuiCom and the coordinating ECCO members responsible for the project. They may call upon external experts (also from outside of ECCO member states and outside IBD related areas) provided that they submit their COI before the start of any working party activities and account is taken of extraneous expenses. Criteria for selection of working party members will primarily depend on academic expertise, but appropriate consideration of gender balance, geographical location, and participation in current or previous guideline projects is expected, to avoid the perception of bias. Inclusion of YECCO members in working groups, or as drivers for the project under appropriate senior guidance, is encouraged. Employees of the Pharmaceutical Industry are explicitly excluded from the systematic literature review or meetings of the Consensus, even as observers.
The development of guideline statements and the supporting text always include a systematic literature search with the appropriate key words using Medline/Pubmed and the Cochrane database. Evidence levels (EL) and grades of recommendation (RG) are attributed according to the Oxford Centre for Evidence Based Medicine. To facilitate the discussion among different working groups and to quantify opinions among all working groups an online guideline platform is used for all guideline projects. Usually, two rounds of online voting will be performed. The first round will take place after finalization of the statements by the topic-focused working groups and will involve all participants of the consensus project. The feedback from the first online voting is used to modify and improve the initial statements in order to reach the highest degree of acceptance at the final consensus meeting. A second online voting round takes place after the revision of the statements and in addition to all the consensus participants all National ECCO Representatives and those ECCO members that applied for this guideline but were rejected due to space limitations are involved. The feedback of the second online voting round will again be used to modify and improve the statements in order to reach the highest degree of acceptance at the final consensus meeting. A final consensus meeting takes place after the second online voting round and all ECCO members that were involved in the guideline should aim to attend this meeting. All statements with more than 80% agreement in the second online voting round do not need any additional voting in the consensus panel meeting. All statements with less than 80% agreement will be voted upon and may be modified according to the feedback of the consensus panel members in order to achieve a higher degree of agreement. Statements with more than 80% of agreement in the final consensus meeting or the second online voting round are accepted as final consensus statements. Those with less than 50% agreement in the final consensus meeting are rejected, as there has been no majority among the experts. Those statements with 50–80% agreement represent a majority vote which results in a downgrading of the recommendation grade.
Germany

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When looking at different treatment guidelines the topics most debated are the following:
for Crohn's disease
- the use of mesalamine for remission induction in mild to moderate Crohn's disease
- the early use of anti-TNF-antibodies in Crohn's disease with or without classical immunomodulators for remission induction
- remission maintenance after remission has been achieved with anti-TNF antibodies
for ulcerative colitis
- remission induction in steroidrefractory disease with anti-TNF-antibodies or calcineurin inhibitors

The topics mentioned above will be discussed with regard to the statements of the German Gastroenterology Association (DGVS) on the basis of the underlying evidence.
Great Britain

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Treatment guidelines are a relatively recent phenomenon and have flourished in tandem with the internet. The content and steer of guidelines have several influences; the quality and availability of clinical evidence, the expert opinion of authors, the targeted readership, the structure in which healthcare is delivered and constraints on healthcare budgets. With influences that evolve like shifting sand, guidelines require regular revision.

The management of inflammatory bowel disease represents a key component of clinical practice for members of the British Society of Gastroenterology (BSG). There has been considerable progress in management strategies affecting all aspects of clinical care since the publication of previous BSG guidelines in 2004, necessitating the present revision.

Key components of the 2011 guidelines worthy of attention as having been subject to re-assessment, and revision, and having direct impact on practice include:

- The data generated by the nation-wide audits of Inflammatory Bowel Disease (IBD) management in the UK in 2006, and 2008.
- The publication of Quality care: Service Standards for the healthcare of people with IBD in 2009.
- The introduction of the Montreal classification for Crohn’s disease and ulcerative colitis.
- The revision of recommendations for the use of immunosuppressive therapy.
- The detailed analysis, guidelines and recommendations for the safe and appropriate use of biological therapies in Crohn’s disease and ulcerative colitis.
- The reassessment of the role of surgery in disease management, with emphasis on the importance of multi-disciplinary decision-making in complex cases.
- The availability of new data on the role of reconstructive surgery in ulcerative colitis.
- The cross-referencing to revised guidelines for colonoscopic surveillance, for the management of metabolic bone disease, and for the care of children with inflammatory bowel disease.
- Use of the BSG discussion forum available on the BSG website to enable ongoing feedback on the published document [http://www.bsg.org.uk/forum (last accessed Oct 2010)]/.

The guideline is intended primarily for the use of clinicians in the United Kingdom, and serves to replace the previous BSG guidelines in IBD, whilst complementing recent consensus statements published by the European Crohn’s and Colitis Organisation (ECCO).
International guidelines provide different interpretations of the evidence regarding benefits and risks regarding several different aspects of medical therapy for ulcerative colitis and Crohn’s disease. The organizers have focused on three different interventions:

1. Use or nonuse of 5-ASA in Crohn’s disease
2. Early use of anti-TNF (top down)
3. Calcineurin inhibitors versus anti-TNF in steroid refractory UC

Representing the U.S. organizations that have published guidelines from the American College of Gastroenterology and the American Gastroenterological Association I will review the current recommendations.

Regarding the use of 5-ASA in Crohn’s disease the American College of Gastroenterology Guidelines published in 2009 concluded that while ileal, ileocolonic, or colonic disease has commonly been treated in clinical practice with oral mesalazine 3.2–4 g daily (EL, grade C) or sulfasalazine for ileocolonic or colonic disease as 3–6 g daily (grade A) new evidence suggests that this approach is minimally effective as compared with placebo (grade A) and less effective than budesonide or conventional corticosteroids (grade A).1 Furthermore, the ACG Guideline concludes that neither sulfasalazine nor mesalazine have consistent maintenance benefits after medical inductive therapy (grade A). Subsequently, a meta-analysis published in the American Journal of Gastroenterology found a trend towards a benefit with sulfasalazine over placebo (two RCTs, RR of failure to achieve remission = 0.83; 95% CI = 0.69–1.00), but no definite benefit of mesalazine over placebo (four RCTs, RR = 0.91; 95% CI = 0.77–1.06). Neither sulfasalazine nor mesalazine were effective in preventing quiescent CD relapse, but in a per protocol analysis mesalamine appeared to reduce risk of relapse (RR = 0.79; 95% CI = 0.66–0.95, NNT = 13). Hence, the authors concluded that “The role of 5-ASAs in inducing remission of active CD and preventing relapse of quiescent CD remains uncertain, and more RCTs are required.”2

The ACG Guidelines beg the question of early use of anti-TNF/top down therapy by stating that “Patients with moderate to severe disease are treated with prednisone 40–60 mg daily until resolution of symptoms... (grade A) and that “anti-TNF monoclonal antibodies, infliximab, adalimumab, and certolizumab pegol, are effective in the treatment of moderate to severely active CD in patients who have not responded despite complete and adequate therapy with a corticosteroid or an immunosuppressive agent (grade A).”1 The AGA Guidance on the use of biologic agents predated the trial regarding early intervention3 and the most recent ACG sponsored meta-analysis did not discuss positioning of biologics compared with corticosteroids with respect to earlier interventions.4
Similarly, neither the AGA or ACG have taken a position on the comparative effectiveness of calcineurin inhibitors with respect to anti-TNF agents for steroid-refractory ulcerative colitis. The ACG guidelines state that “failure to show significant improvement within 3–5 days is an indication for either colectomy (Evidence B) or treatment with intravenous cyclosporine (CSA; Evidence A) in the patient with severe colitis” and that “Infliximab may also be effective in avoiding colectomy in patients failing intravenous steroids but its long-term efficacy is unknown in this setting (Evidence A).” These guidelines were published prior to the reports on the recent comparative trial by Laharie.

References:


Japan

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Diagnostic and therapeutic approaches to inflammatory bowel disease (IBD) have significantly changed in Japan recently. In addition, the number of IBD patients has been increasing in Japan. We have therefore published a set of clinical practice guidelines for Crohn’s disease (CD) in 2011 and ulcerative colitis (UC) in 2006. These guidelines aim to provide appropriate clinical indicators to Japanese practitioners to improve the outcomes of IBD patients. These guidelines are based on global literature-based evidence as well as evidence from Japan.

The Japanese guidelines were developed based on the existing evidence with integration of consensus of Japanese experts evaluated with the Delphi method. The criteria for recommendation grade were also determined by the level of evidence as well as the consensus of the experts. It is a distinct feature of the Japanese guidelines to disclose this process explicitly. This recommendation rating of the Japanese guidelines is thus useful to fill the gap between evidence and daily clinical practice.

The statements in the Japanese guidelines mostly reached similar conclusions with those published in Europe and North America, based on the same evidence. However, some statements in the Japanese guidelines reflect Japanese perspectives and evidence. For example, in the CD guidelines, 1) The Japanese guidelines recommend contrast radiographic examinations for diagnosis of the small intestinal lesions rather than MRI and CT. 2) Elemental diet and cytapheresis are more emphasized in the Japanese guidelines. 3) 5-aminosalicylate is not recommended in the western guidelines because of its minimal effect on CD, but it has a role in the management of CD in the Japanese guidelines because of its safety profile. It should be also emphasized that the diagnostic criteria of CD established and widely used in Japan were employed. In the UC guidelines, cytapheresis is also appreciated as an alternative therapy for steroid-refractory patients. While oral tacrolimus and infliximab has been widely used in Japan for steroid-refractory patients, those agents are not included in the current guidelines. The UC guidelines are now under the process of revision to include those updates.

Since the Japanese guidelines for CD are primarily based on global literature-based evidence, most of the clinical indicators in them are consistent with those in other guidelines from the western world. Meanwhile, there are some distinctly different statements in the Japanese guidelines reflecting Japanese standard clinical practice, evidence, and the opinions of Japanese experts.
Session VI

Adverse events
Aminosalicylates and corticosteroids

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Aminosalicylates, mainly mesalazine (5-ASA), are recommended for the induction of remission and maintenance therapy in ulcerative colitis. In Crohn’s disease, the efficacy has not been proven. In general, the treatment with 5-ASA is well tolerated and the adverse event rate of 5–10% is comparable to placebo in clinical trials. The safety profile is superior to the old aminosalicylate prodrug sulfasalazine, which had a significantly higher incidence of intolerance reactions including allergic rashes. Only in rare cases, nephrotoxicity such as renal impairment and interstitial nephritis have been associated with 5-ASA. Therefore, before and during treatment with 5-ASA the renal function should be assessed.

Glucocorticosteroids are highly effective in inducing remission in acute exacerbations of both ulcerative colitis and Crohn’s disease. Due to their broad indication in other medical entities glucocorticosteroids are widely available and cheap. However, their use is limited by the high incidence and the potentially serious nature of adverse events. In addition, patients may develop corticosteroid dependence. The numerous adverse events, particularly at high doses and prolonged treatment include opportunistic infections, diabetes mellitus, hypertension, ocular effects (glaucoma and cataracts), psychiatric complications, hypothalamic-pituitary-adrenal axis suppression, bone density loss and increased fracture risk. Besides, if corticosteroids are not appropriately tapered after a longer duration of treatment an adrenal insufficiency may develop. As a consequence of this toxicity profile, one primary goal of treatment in inflammatory bowel diseases is the corticosteroid-free remission. In an attempt to limit systemic side effects, rapidly metabolized corticosteroids such as budesonide and beclomethasone dipropionate have been introduced. Their high first-pass metabolism and lower systemic availability may result in a better safety profile.
Immunosuppressants

Matthias Schwab
Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany

Variation in drug disposition and response among patients is a major concern associated with many therapeutical agents used in all disciplines of medicine. A broad variety of individual factors may influence pharmacokinetics, commonly referred to the acronym ADME (drug absorption, distribution, metabolism, and elimination) of a drug and must, therefore, be taken into account when determining dosage for a given patient. Drug concentration at its target will, in many cases, represent a mere fraction of the systemic concentration. Active transport processes, however, may influence local target concentrations. It has become increasingly clear that hereditary variance in drug metabolizing enzymes and drug transporters can exert considerable influence on drug concentrations of immunosuppressants used in treatment of patients with IBD. However, in addition to inherited variants, many other non-genetic factors (e.g. age, sex, weight, body fat, alcohol consumption, concomitant drugs, nutritional status, liver and renal function, cardiovascular function, environmental pollutants) possibly influence ADME processes of drugs, thereby contributing to drug response and/or drug safety. There is increasing evidence that a more patient-tailored therapy of IBD patients using immunosuppressants like thiopurines, methotrexate and calcineurin inhibitors (e.g. tacrolimus) will improve therapeutic outcome by avoidance of severe adverse drug reactions. Recently developed -omics approaches like transcriptomics and metabonomics will be helpful to identify further putative targets for better prediction of drug response. Epigenetic aspects (e.g. DNA methylation, miRNA) need to be considered more intensively in the future.
Anti-TNF

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The well established class of TNF inhibitors for treatment of inflammatory diseases including IBD has grown up. Records from case series and randomized controlled trials as well as registries state that treatment is effective and safe. However, there are concerns regarding adverse events which may discourage early administration. Other drugs, ranging from 5-ASA, glucocorticoids up to classical immunosuppressants do have their own risks and benefits. We have come aware that combined treatment bears the highest risk of untoward adverse events, especially with regards to opportunistic infections.

Acute adverse events in anti-TNF treatment can be divided in allergic reactions with an acute and delayed onset, infectious complications in relation to the underlying disease and without and last but not least the unresolved question of tumor induction and progression.

Allergy is mostly related to the immunogenicity of the antibody. Chimeric antibodies are more prone to this complication than completely humanized TNF blockers but the protein in itself may account for an excess rate of events even with the latter preparation. The immediate type I allergy may range up to allergic shock whilst delayed type IV allergy may present with lymphocyte dependent skin reactions. Rarely serum sickness disease as a type III allergy is seen.

Major drawbacks are the infectious complications especially seen during double or triple immunosuppression. An unrecognized infectious complication in IBD may deteriorate, e.g. abscess formation leading to uncontrolled sepsis. Other infectious diseases may even occur in non IBD patients, tuberculosis for instance or CMV colitis. Differences between these two situations are not as strict as they may pretend to be.

Defining the role of TNF inhibitors in tumor development and propagation is still on the row. Troublesome has been the occurrence of lymphoma including hepatosplenic T-cell lymphoma. But we have to acknowledge that purine analogues do have an incremental risk of lymphoma development, too. A new finding is the risk of melanoma associated with the administration of TNF blockers, while purine analogues increase the risk of non melanoma skin cancer. This fact has already been implanted in patient surveillance.

"Nothing is for free", this also holds truth for blocking TNF. The clinician has to decide whether benefits outweigh the risks of treatment.
Session VII

Treating microbiota and/or the barrier
State-of-the-Art Lecture

New treatments for IBD

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Crohn’s disease represents a life-long condition that is characterized by chronic progression with development of non-inflammatory complications (e.g. stenoses, fistula, abscesses). Only few patients present with uncomplicated courses in which phases of symptomatic activity intervene with a complete remission. Most patients develop an ongoing, chronic inflammatory activity (as defined on the level of the immune system) which persists even if a symptomatic remission can be induced and maintained by therapy. Anti-TNF therapies have been demonstrated to alter the course of disease (i.e. induce mucosal healing and reduce CD-specific hospitalizations and surgeries). However, these agents are often used late in the disease career and therefore in patients who have already developed some non-inflammatory complications. Moreover, it has become clear that the use of the presently available antibodies has a long-term efficacy of well below 50%. Alternatives have to be therefore developed, both with regards to the use of existing agents and the development of drugs representing novel mechanisms of efficacy. The course of ulcerative colitis is less well analyzed. However, in main aspects these observations in Crohn’s disease can be transferred to patients with ulcerative colitis.

Several novel anti-inflammatory therapies have successfully completed clinical development. The most promising strategies that very soon will be available include the blockade of adhesion molecules (mainly the $\alpha_4\beta_7$ – MadCam complex), blockade of interleukin-6 and inhibition of cytokine-receptor associated janus kinases. The specific blockade of adhesions molecules on the level of the gut holds the promise that very little systemic side effects may be induced. This particular advantage for the $\alpha_4\beta_7$ integrin antibody vedolizumab that is suggested by a particularly large clinical trials program has to be further corroborated by the clinical experience after market introduction.

IL-6 blockade through various monoclonal antibodies and the blockade of janus kinases through tofacitinib produce substantial systemic immunosuppression including the typical side effects which comprise as a main risk the increased occurrence of infections. IL-6 blockade with some agents allows the dissection between trans-signaling and classical signaling offers a strategy that could lead to elimination of chronic inflammatory activity without the side effects of systemic immune suppression.

In contrast, strategies that specifically inhibit T-cells have failed development.
However, before embarking on novel therapies, the present use of anti-TNF for the therapy of IBD should be optimized. Anti-TNF therapy is often used too late in the course of disease to still avoid the formation of anatomical damages. Secondary failures to anti-TNF have to be analyzed to decide whether a dose increase or intensification, a switch between anti-TNF agents or a change in the substance class for therapy is more beneficial to the patient, provided that uncontrolled inflammation is causing the clinical problems. Novel anti-TNF antibodies like golimumab that show less immunogenicity than older agents may become important tools in this regards.
Antibiotics

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Crohn’s disease (CD) is a chronic inflammatory bowel disease characterized by an altered immunological response to the intestinal commensal bacteria. Dysbiosis is considered to have a preeminent role in CD pathogenesis by inducing an abnormal immune response in genetically susceptible individuals. Many data support the bacteria involvement in IBD, mainly in CD; the most important datum is that the luminal content is necessary for causing the intestinal inflammation. CD lesions do not appear when the stools are diverted from the gut, whereas restoration of bowel continuity or infusion of faecal material into the bypassed intestine rapidly results in recurrence of inflammation. Increase of aggressive types of bacteria and decrease of protective strains is a characteristic of the CD intestinal microbiota. Recently the discovery that E. coli adhere better to the epithelial cells of the CD ileum in comparison with control, has further supported the role of bacteria in CD pathogenesis. Moreover some of the genes involved in CD predisposition play a role in the intestinal immunological response to bacteria. Consequently the involvement of bacteria in CD inflammation provides the rationale for including antibiotics in the therapeutic armamentarium.

While antibiotics are frequently employed for treating CD, on the flip side of the coin recent epidemiological studies have shown that early life exposure to antibiotics might alter the development of the host microbiome and influence CD risk. The role of antibiotics as primary or adjunctive treatment of active, uncomplicated CD has not been clearly demonstrated and their use is controversial. A recent meta-analysis suggests that antibiotics may be effective as primary therapy of CD, but guidelines do not recommend their use except for the treatment of septic complications of CD, symptoms attributable to bacterial overgrowth, or perianal disease. In the hope of curing CD, antibiotics active against atypical Mycobacteria, suspected to be the primary cause of CD, have been tested, with conflicting outcomes. The most important study employed the antibiotic clofazimine together with clarithromycin and rifabutin, against placebo, for up to 2 years. Only a modest non significant advantage of antibiotics over placebo in reducing CD relapse was registered at every observation time point during the 2-year period.

Several randomized clinical trials have been performed employing metronidazole and/or ciprofloxacin for induction of CD remission. The results of the trials have indicated that metronidazole is efficacious in active Crohn’s colitis and ileo-colitis, but not in small bowel location. Five randomized controlled studies evaluating the efficacy of ciprofloxacin, alone or in association with metronidazole, in patients with active CD, have shown uncertain results. Patients with colonic involvement get more benefit from antibiotics, probably because of the high concentration of bacteria in the colon. Metronidazole and ornidazole have been employed for preventing 1 year post-surgical recurrence. They were shown to significantly reduce the clinical recurrence rate at 1 year, but more than 30% of patients in the antibiotic group discontinued therapy because of side effects. In fact the prolonged administration of antibiotics is frequently burdened by an elevated number of systemic side effects. For this reason rifaximin, a non-absorbable antibiotic with a high safety profile has been used for treating mild to moderate CD.
402 patients were randomized to receive rifaximin-EIR 400, 800, 1200 mg or placebo twice daily for 12 weeks. The results showed that rifaximin-EIR 800 mg twice daily was significantly superior to placebo in inducing remission (62% vs 43%). Colonic location, CRP elevated value and early disease appeared to be associated with a higher response to the antibiotic therapy. Overall, the safety profile of rifaximin-EIR was good, suggesting that rifaximin could be administered for a long period of time. In conclusion there seems to be a subgroup of patients with colonic disease and high CRP value who can respond to antibiotics. Nitroimidazole antibiotics seem to be effective in decreasing both endoscopic and clinical recurrence rates after surgery, but their long-term use is complicated by an elevated number of AEs. Treatment of patients with mild and moderate CD with rifaximin seems promising, but further larger studies are needed.

References:


The overwhelming number of bacteria and the diversity of the enteric flora have been demonstrated to have significant impact on the health and disease status of the intestines. The barrier function of the mucosa plays a key role in this ecological system. In view of the impact of intestinal bacteria, it is intriguing to intervene with its composition in order to exert therapeutic effects.

Therapeutic intervention on the enteral flora can be accomplished by several routes. Diet and dietary supplements such as fibers can act as prebiotics and thus alter the bacterial pattern. A more specific approach is the use of defined bacteria, which can be ingested as single microorganism or in combination.

Clinical studies have been performed using all kinds of bacteriotherapy but randomized controlled trials are only with defined probiotics existing. Predominantly three areas of intestinal disease are in the focus of investigations: functional bowel disorders (IBS), inflammatory bowel diseases (IBD) and infection with Clostridium difficile (CDI). Specific and well defined probiotics have shown convincing therapeutic effects in ulcerative colitis and pouchitis but have failed in Crohn’s disease. Bacteriotherapy of chronic pouchitis and maintenance of remission of ulcerative colitis have reached the level of recommendations of international guidelines (European Crohn’s and Colitis Organisation, ECCO). Varrying results in different disease entities may point to the possibility of individual approaches of bacteriotherapy to treatments.

Valid trials with prebiotics are nearly lacking. Plantago has demonstrated effects in relapse prevention. In addition, the combination therapy with prebiotics (Inulin) and probiotics (symbiotic treatment) has been tested in active ulcerative colitis with promising results.

In light of some reports of even fatal outcomes safety has to be strictly considered when comparing the different routes of bacteriotherapy. As yet diets and prebiotics have not shown any serious adverse effects. The situation for probiotics is very inconsistent. Few probiotics (e.g. E. coli Nissle) have been gone through all the processes for becoming approved as pharmaceuticals and have thus extensive safety records. Other probiotics, particularly combinations did less well perform and therefore safety concerns still remain.

There are wide differences in knowledge of microbiological and immunological effects. Only some specific bacteria and one combination have been investigated sufficiently. All the varying results in different disease entities may point to the possibility of individual approaches of bacteriotherapy to treatments. Head-to-head comparisons in one indication are currently not available.

In summary in some diseases good evidence of therapeutic efficacy is existing, but altogether bacteriotherapy needs far more research work for final statements.
The lecithin story: Mucosal protection by phosphatidylcholine

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The colonic mucus serves a first barrier towards invasion of commensal bacteria in stools to prevent inflammation. One essential component of intestinal mucus is phosphatidylcholine (PC) which represents more than 90% of the phospholipids in mucus. It is arranged in lamellar structures as surfactant-like particles which provide a hydrophobic surface on top of the hydrated mucus gel to prevent the invasion of bacteria from intestinal lumen. In ulcerative colitis (UC), the mucus PC content is reduced by 70%, irrespective of the state of inflammation. Thus, it could represent an intrinsic primary pathogenetic condition predisposing to bacterial invasion and the precipitation of inflammation. Since PC was shown to be mainly secreted by the ileal mucosa from where it is assumed to move distally to the colon, the PC content along the colonic wall towards the rectum gradually thins, with the least PC content in the rectum. This explains the start of the clinical manifestation of UC in the rectum and the expansion from there to the upper parts of the colon. In three monocentric clinical trials, when missing mucus PC in UC was supplemented by an oral, delayed release PC preparation, the inflammation improved and even resolved after a 3-month treatment course. In a recent multicentric randomized clinical phase IIB trial those previous results could be confirmed in regard to improvement of clinical activity at 3.2 g LT-02 as well as achievement of mucosal healing.

The data indicate the essential role of the mucus PC content for protection against inflammation in colon.
Trichuris suis in IBD

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Inflammatory Bowel Disease (IBD) has emerged during the last century and is considered a "civilization disorder" with genetic background but necessary environmental stimuli to manifest itself. One such environmental stimulus has been suggested to be hygiene or more specifically the lack of helminth infection during childhood and beyond.

Some but not all epidemiological studies suggested that helminth infection in childhood protects against development of IBD in later years. This is, however, hotly debated due to methodological problems. In animal models of IBS helminths have shown protective effects and changed bacterial flora in the gut.

Based on these speculations and observations the group of Weinstock and Summers tested colonization with Trichuris suis in patients with IBD. After initial positive results in 7 IBD patients a series of 29 patients with active Crohn’s Disease (CD) were treated with repeated (3 weekly) application of 2500 ova of T. suis. 79% had significant response and 72% achieved remission (CDAI < 150). They furthermore conducted a double-blind placebo-controlled study in 54 patients with active Ulcerative Colitis (UC) using 2500 ova every two weeks. Response occurred in 43.3% of T. suis-treated patients vs. 16.7% of placebo patients. Blinded crossover resulted in 56.3 vs. 13.3% response. It was concluded that repeated administration of 2500 ova of T. suis is safe and likely efficacious to treat IBD.

Early trials in other disorders such as multiple scleroses and asthma or allergic rhinitis revealed mixed results but be approach is still going on.

Based on the results mentioned further trials in IBD were started. A small placebo-controlled study of 36 patients with CD using 500, 2500, 7500 ova did not find adverse symptoms. A larger multicenter double-blind placebo-controlled study of 250, 2500 and 7500 ova or placebo is running in Europe. An interim analysis of 120 patients revealed a pooled response rate in all groups (CDAI drop > 100) of 60% at week 12 (CDAI drop > 70: 68%), and a remission rate of 47%. CDAI decreased from 282 to 104 in 12 weeks. No important risks have been thus far identified. Meanwhile 209 patients have been randomized (status 06.03.2013). A similar trial is running in the US.

The results of these large trials remain to be awaited in order to clarify if further development of this concept is feasible and promising.
State-of-the-Art Lecture

Stem cell therapy for IBD?

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Recent studies have expanded our knowledge of gastrointestinal stem cell biology. We developed a novel culture method that maintains Lgr5 colonic stem cells in vitro. The crypt cells formed a round cystic structure consisting of epithelial monolayer of multilineage cells and could be propagated without losing their properties. Importantly, expression of Lgr5 was significantly up-regulated and then constantly maintained for a long time period. Moreover, successful, long-term engraftment was observed even with the transplantation of organoids that were derived from a single Lgr5 colon stem cell after extensive in vitro expansion. Transplanted cells readily integrated into the colonic tissues covering the area that lacked epithelium, and accelerated the recovery of recipients from acute colitis. Donor-derived cells constituted single-layered epithelium forming self-renewing donor-derived crypts that were functionally and histologically normal. We developed human colonic epithelial cell culture from normal and IBD patients.

Our data for the first time demonstrate that adult stem cell therapy by in vitro expansion and transplantation of gastrointestinal stem cells could be an option for patients with severe gastrointestinal epithelial injuries such as IBD in humans.

Using our intestinal 3D culture system, we have developed an in vitro experimental model that mimics P-gp-mediated intestinal drug transport in vivo. We are also constructing the real time imaging of interactive movement between colonic epithelial cells and immune cells for application in mucosal immunology for studying the basic mechanism for IBD.
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POSTER ABSTRACTS

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Pre-treatment with non-absorbable glucids and bovine glycomacropeptide ameliorates 5-fluorouracil-induced intestinal mucositis in rats

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Introduction: Intestinal mucositis is a common side-effect of high-dose chemotherapy. The nutraceuticals goat’s milk oligosaccharides (GMOS) and bovine glycomacropeptide (GMP) may be useful in this condition, given their intestinal anti-inflammatory properties.

Methods: Female Dark Agouti rats were treated with the products at the dose of 500 mg/kg for 7 days and then received an intraperitoneal injection of 150 mg/kg 5-FU. Animals were killed on day 14 for assessment of intestinal damage and repair.

Results: Compared to controls, 5-FU produced a significant loss of body weight, which was reduced in the GMOS group (145.0 ± 1.0 vs. 152.5 ± 2.2 g), and an increase of the ileon weight-length ratio, reversed by GMOS as well (45.1 ± 1.0 vs. 55.3 ± 5.1 mg/cm, p < 0.05). Colonic alkaline phosphatase activity was increased in the 5-FU group compared to the control (46.6 ± 4.6 vs. 36.4 ± 3.5 U/mg protein), an effect that was prevented by GMOS (29.1 ± 1.4 U/mg protein, p < 0.01). Colonic myeloperoxidase activity showed an 80% decrease in the 5-FU group (3.6 ± 2.4 vs. 18.8 ± 7.5 mU/mg protein) that was only slightly reduced by GMOS (4.5 ± 4.1 mU/mg protein). Other biochemical parameters assessed in the ileum were the activity of the disaccharidases sucrase, lactase, and maltase, all of which were similarly decreased in the 5-FU group compared to the control group, and normalized by both GMOS and GMP. Finally, we determined the ileum expression of MUC3 and MUC4 by real time PCR, showing an increase by the administration of 5-FU and reversion by both treatments.

Discussion/Conclusion: GMP and especially GMOS exert beneficial effects in the 5-FU mucositis model.
Alternative technical approach to quantify bacterial uptake by intestinal epithelial cells

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Introduction: Crosstalk between commensal bacteria and intestinal epithelium is an important issue in inflammatory bowel disease and other intestinal conditions. It is therefore interesting to be able to measure parameters such as bacterial invasion in a rapid and efficient way.

Methods: IEC18 and CACO-2 cells, and E. coli K12 and LF82 (AIEC) strains (gently provided by Dr. Arlette Darfeuille-Michaud) expressing GFP were used. Confluent monolayers grown in 12 well plates were infected with a multiplicity of infection (MOI) of 100 bacteria/epithelial cell in regular medium (DMEM). Subsequently, monolayers were washed three times with HBSS and fresh regular medium supplemented with, kanamycin 100 μg/ml, penicillin 500 U/ml and streptomycin 0.5 mg/ml for 1 h to eliminate extracellular bacteria. Then cells were washed again and collected by trypsinization, analyzed by FACS and expressed as percentage of FL1-GFP+ cells. Confocal microscopy was used to confirm intracellular localization.

Results: LF82 but not K12 were shown to locate intracellularly and to reach substantial invasion levels (50–60%) in a matter of a few hours. The main variable was bacterial exposure time, with less impact of both MOI or antibiotic treatment time. Reproducible results were obtained after as little as 4 h. This setting was applied to the study of several experimental treatments for inflammatory bowel disease, namely fructo-oligosaccharides (FOS), quercetin and rutin. Unlike both flavonoids, FOS addition at a concentration of 1 mg/ml up to 20 mg/ml, 24 h before and during bacterial infection inhibited cell invasion in a concentration dependent fashion, 18.6 ± 8% and 77.0 ± 1.15%, respectively.

Discussion/Conclusion: Here we report a cost-effective, practical and reproducible assay for bacterial invasion. It may be used as an alternative to the classical and widely used gentamicin protection one, which demands more material, time and personnel resources. We are currently adapting the method to regular bacterial translocation.
Predicting mucosal healing in Crohn’s disease using practical clinical indices with regard to the location of active disease

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Introduction: Only a few reports have examined the relationship between balloon-assisted enteroscopy (BAE)-based mucosal lesion severity in Crohn’s disease (CD) with clinical variables such as serum levels of disease activity markers and Crohn’s Disease Activity Index (CDAI). We analyzed whether clinical variables are useful to predict mucosal healing (MH) in various CD types.

Methods: A total of 156 CD patients who underwent BAE were enrolled. The “ileum group” included patients with Simple Endoscopic Scores for CD (SES-CD) ileum score greater than the sum of the SES-CD colon scores (n = 124), whereas the “colon group” included patients who had the ileum score greater than the sum of SES-CD colon scores (n = 56). Endoscopic findings were assessed by both SES-CD and Modified Rutgeerts Score (MRS).

Results: The colon group had better correlativity in ROC analyses for the prediction of MH. C-reactive protein (CRP) was the most accurate marker for predicting MH in the colon group. The MH index obtained from logistic regression analysis was the most accurate marker in the ileum group. A decision-tree model using CRP and serum albumin levels was built; 78.2% (122/156) of all patients and 76.1% (54/71) of patients who underwent second BAE attempts were correctly classified.

Discussion/Conclusion: For predicting MH, CRP levels are useful in dominant colonic CD and the MH index is useful in dominant ileal CD. For clinical settings, a decision-tree model can be used as a guide to predict MH probability.
Elafin is upregulated by *E. coli* Nissle 1917 flagellin via TLR5 and NF-kappaB

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**Background:** Elafin is an inflammatory induced serine antiprotease produced by various epithelia, such as the gastrointestinal tract. Its main function is to neutralize the two proteases human neutrophil elastase and proteinase-3, once secreted during inflammation leading to tissue destruction. Additionally, elafin possesses antimicrobial properties against different bacteria and viruses. Recent studies found elafin (but not human neutrophil elastase) induction to be attenuated in active versus inactive Crohn’s disease as compared to ulcerative colitis, the two major forms of inflammatory bowel diseases. Moreover, elafin overexpression was demonstrated to be protective in a murine model of colitis. In this study, we evaluated the regulation of elafin by intestinal bacteria and their components, NF-kappaB pathway as well as TLR5 *in vitro*.

**Methods and Results:** Elafin expression was strongly upregulated in the human colon adenocarcinoma cell line LS174T stimulated with different apathogenic and in part probiotic bacteria, especially with *E. coli* K-12 and *E. coli* Nissle 1917. This *E. coli* K-12 and *E. coli* Nissle 1917 triggered elafin induction was missing following a coincubation with the anti-human TLR5 polyclonal antibody Pab-hTLR5 and NF-kappaB blocker Helenalin and Bay 11-7082. Moreover, treatment with different *E. coli* Nissle 1917 wild type and mutant strains showed elafin induction to be inhibited or even blocked by the absence of flagellin.

**Conclusions:** Different intestinal bacteria, especially the probiotic strain *E. coli* Nissle 1917, are effective to induce elafin. The bacteria triggered increase of elafin expression is regulated by TLR5 and NF-kappaB and, moreover, in case of *E. coli* Nissle 1917 it depends on flagellin. Probiotic *E. coli* Nissle 1917 is already successfully used to treat ulcerative colitis. It may be speculated that this effect is mediated amongst others by the induction of protective elafin expression.

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Crohn's disease masked by median arcuate ligament syndrome

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Introduction: Median arcuate ligament syndrome (MALS) or celiac axis compression syndrome is an uncommon cause of mesenteric ischemia. Median arcuate ligament usually passes superior to the origin of the celiac axis. Celiac trunk is compressed by the median arcuate ligament during expiration [1]. The majority of patients are observed with symptoms weight loss and abdominal pain. Celiac artery compression with Doppler ultrasound, CT angiography, selective catheter angiography and magnetic resonance angiography can be ascertained. After the disease is diagnosed, surgery is performed to relieve the compression [2].

Methods: Hereby, we describe a 26-year old male patient. Before applying our hospital, he was investigated at another medical center. Abdominal ultrasound, upper gastrointestinal endoscopy and laboratory tests were performed. All these tests were within normal range. He was admitted to the gastroenterology department of our hospital due to abdominal pain, loss of appetite, watery stool (once a day) and weight loss (14 kilos) during the last four years. Physical examination revealed abdominal tenderness and pain. On color Doppler ultrasonography, it was observed that the velocity of celiac artery increased. Computed tomography (CT) enterography showed narrowing in the proximal part of the celiac artery due to median arcuate ligament compression and diagnosis of MALS was confirmed. The patient adamantly refused surgery and medical therapy was performed. Colonoscopy was performed on the patient who was suffering from ongoing diarrhea and revealed multiple nodules in the terminal ileum. The diagnosis of Crohn's disease was confirmed with biopsy of the terminal ileum.

Results: During the following period after therapy, the patient's clinical status improved by taking mesalazine and budesonide.

Discussion/Conclusion: Patients complaining about vomiting, weight loss and abdominal pain should be considered in terms of many diseases including MALS. Atherosclerotic disease and celiac artery embolism should be considered in the differential diagnosis of MALS [3]. In addition, MALS as in our case with Crohn's disease can be observed in the same patient.
References:


Induction of dextran sulfate sodium colitis in germ-free conditions

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Introduction: The etiology of inflammatory bowel diseases (IBD) is largely unknown, but appears to be perpetuated by uncontrolled responses to antigenic components of the endogenous flora. Our aim is to study the effect that causes the presence of bacteria in dextran sulfate sodium (DSS)-induced colitis.

Methods: Conventional (43) and germ-free NMRI mice (GF) (40) were used. Colitis was induced by adding 0–4% DSS to drinking water. Some GF mice also received a sterile bacterial homogenate (SBH) in the diet. After 7 days, large intestine status was evaluated by macroscopic scoring, myeloperoxidase (MPO) and alkaline phosphatase (AP) activity. Gene expression analyses by RT-PCR were conducted and mice spleen mononuclear cells (MSMC) cultures were assayed for pro-inflammatory cytokine content by ELISA.

Results: Colitis was induced in all groups, manifested as a measurable loss of body weight, blood in the stool, diarrhea, and general deterioration of health. However, there were notable differences in the phenotype. The macroscopic score of the large intestine was lower in GF and the colonic weight/length ratio was not increased. There was no neutrophil recruitment to the inflammatory site in GF animals, as measured by MPO activity, and colonic AP activity was diminished. Pro-inflammatory cytokine production was reduced in GF MSMC cultures and, more importantly, it was essentially unresponsive to concanavalin A (ConA) stimulation. Colonic GF mRNA levels were all diminished. The addition of SBH in the diet showed no major changes. Although not tested formally, the general appearance of colitic GF animals suggested a massive blood loss.

Discussion/Conclusion: Enteric bacteria are essential for the development of normal DSS-induced colitis. Our results suggest that in GF conditions DSS evokes a primary epithelial injury but the subsequent immune response is absent. Apparent aggravation of animal status may be the result of blood loss rather than colitis.
Two cases of newly developed ulcerative colitis following clinical and endoscopical remission of acute bacterial colitis

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Introduction: Although the causal relationship of infectious pathogens to initiation of ulcerative colitis is debatable, some case reports demonstrated the development of ulcerative colitis following acute bacterial enterocolitis. Moreover, recent epidemiologic cohort studies suggested that the risk of inflammatory bowel disease was greater in those with a previous episode of infectious diarrhea. However, it may be unclear whether ulcerative colitis was actually triggered by bacterial infection or became overt by coincidental infectious diarrhea. Therefore, the sequential colonoscopic features as well as clinical manifestations following infectious enterocolitis can be helpful to demonstrate the development of ulcerative colitis triggered by infection.

Case: Two young male patients in their 30’s presenting with acute abdominal pain and diarrhea visited our gastrointestinal clinic in June 2009 and March 2010, respectively. One patient drank from a fountain and the other had seafood a few days before diarrhea. On colonoscopies, findings of non-continuous extensive colitis with rectal sparing were noted. After antibiotics therapy, complete clinical and endoscopic remissions were achieved within one to three weeks. Six to twelve months later, however, they revisited our clinic presenting with recurrent bloody diarrhea, showing new development of definitive findings of proctitis or rectosigmoiditis, which were endoscopically different from initial findings. Histology of the colonic mucosa revealed chronic inflammation with cryptic abscess and basal lymphoplasmacytosis. Stool cultures were negative for bacterial and parasitic infections. *Clostridium difficile* toxin assays were also negative. Diagnoses of distal ulcerative colitis were made, and oral and local mesalamine therapy was initiated daily. Their symptoms improved promptly and they have been on maintenance therapy.

Conclusion: The sequence of colonoscopic findings as well as clinical symptoms in these cases suggests that bacterial infection may contribute to the development of ulcerative colitis. This supposition should be supported by large-scale prospective clinical trials of patients with acute infectious enterocolitis.
Regulation of \(\alpha\)-defensin expression by inflammatory processes and bacteria

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Background: In the small intestine the Wnt pathway is important for stem cell proliferation but also Paneth cell differentiation. Paneth cells are predominately located at the bottom of small intestinal crypts and generate antimicrobial peptides. Their most abundant innate defence molecules, the \(\alpha\)-defensins HD5 and HD6, are important players in regulating gut microbiota. Their expression is partly controlled by the Wnt pathway and is decreased in ileal Crohn’s disease (CD). In patients with small intestinal CD we found impairments of important Wnt pathway components. Additionally it is well accepted that intestinal microbes can influence CD development. Here we want clarify the role of bacteria in Wnt-mediated defensin expression. Furthermore, we analyze the influence of inflammatory processes in this setting aiming to improve our understanding of microbial and inflammatory contributions in CD pathogenesis.

Methods: Modulation of defensin expression is investigated using reporter gene assays in Hek293 cells under different conditions. We treated transfected cells with stimulated peripheral blood mononuclear cell (PBMC) supernatant or heat-killed bacteria. Future experiments will include stimulation of fresh intestinal biopsies and subsequent mRNA analysis.

Results: Stimulated PBMC supernatant can increase Wnt activity (Topflash) and to a smaller extend Paneth cell \(\alpha\)-defensin expression. In first experiments bacteria show no influence in cell lines lacking TLR2 and NOD2.

Conclusion and further experiments: Inflammatory processes can impact HD5 and HD6 expression. Additional research on regulatory cytokines and downstream factors could elucidate the mechanism. Different cell lines or biopsies and UV-killed bacteria will be used to study the impact of microbes in this context for gaining new insights into the regulation of the gut-microbe homeostasis.

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The protective effect of *Echinacea spp.* (*Echinacea angustifolia and Echinacea purpurea*) in rat colitis model induced by acetic acid

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**Introduction:** Ulcerative colitis is a chronic inflammatory condition of the colon and cytokines (such as TNF-α, IL-1β and TGF-β) are crucial components of these inflammatory pathways. New therapeutic strategies are awaited for better clinical outcome of ulcerative colitis and less adverse effects. That’s why alternative therapies such as herbal remedies are increasingly being used for the treatment of ulcerative colitis. Hence, the present study was undertaken to evaluate the protective effect of *Echinacea spp.* on experimental colitis model induced by acetic acid in Wistar albino rats.

**Methods:** Acute colitis was induced by the intrarectal administration of acetic acid. Rats were grouped into 4 as control, Echinacea, Echinacea-colitis and colitis. TNF-α, IL-1β and TGF-β were measured. Histopathological comparison of the groups was also performed.

**Results:** Disease Activity Index (DAI) was significantly higher in colitis group compared to control, Echinacea and Echinacea-colitis groups (p < 0.001). No significant difference between DAI of control, Echinacea and Echinacea-colitis group (p > 0.07). The inflammatory mediators, TNF-α and IL-1β were elevated in colitis group compared to other groups (p < 0.007, < 0.001 respectively).

**Discussion/Conclusion:** Echinacea may possibly have some therapeutic usefulness in the management of ulcerative colitis.
Genetic susceptibility to violations of mucosal barrier in ulcerative colitis and Crohn’s disease

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Background: The mucus layer play important role in host innate defense, regulation of secretion and absorption processes, maintaining colonization resistance, which compose the integrity of protective mucus barrier in the large intestine. Stimulation of Toll-like receptors (TLRs) responsible for recognizing of pathogen-associated molecular patterns by bacterial lipopolysaccharides leads to expression of pro-inflammatory cytokines and induce immune response.

Aim: To characterize genetic susceptibility to affected innate immune response, mucin genes expression in the large intestine and influence of gut microbiome on the development of ulcerative colitis (UC), Crohn’s disease (CD).

Materials and methods: 98 patients with UC and CD, and 30 healthy controls were examined. Polymorphism of TLR3 (Phe412Leu), TLR4 (Asp299Gly) mRNA were determined by reverse-transcription polymerase chain reaction with electrophoretic detection in 3% agarose gel. Histological analysis of colon mucosa was done by standard methodologies (hematoxylin-eosin, alcian blue at pH 1.0 and 2.5, PAS-reaction). Immunohistochemistry was performed using monoclonal mouse antibodies raised against mucins (MUC2, MUC3, MUC4) and TFF3. Changes of fecal microbiota reflected in short chain fatty acids (SCFAs) were determined by gas-chromatography.

Results: 35 (87.5%) CD and 42 (72.4%) UC patients had TLR3, TLR4 polymorphism. Positive interaction between risk genes and the severity of UC (OR = 2.61; p = 0.005) and CD (OR = 3.62; p = 0.001) was determined. The moderate staining of MUC2 and MUC3 (55.0% and 32.5%; p = 0.03), and high expression of MUC4 and TFF3 in the colon mucosa were observed in all patients with CD. The intensive labeling of MUC4 and TFF3 occurred more often (45.0%, p = 0.03 and 52.5%, p = 0.05) in patients with CD. The level of expression of mucins in all patients with UC was low, up to its complete absence (51.7% and 48.3% cases; p = 0.01). TFF3 expression had high and medium staining intensity in patients with UC. The most pronounced alteration of mucins expression observed in patients with severe UC and CD. SCFAs assessment showed deep imbalance of aerobic and anaerobic flora, which correlated with severity of disease.

Conclusions: Genetic susceptibility to changes of colon microbiota, different type of mucins synthesis, secretion and expression were found in patients with UC and CD. The expression of mucin MUC2, MUC3, MUC4 and TFF3 was correlated with the activity of disease and the extent of the inflammatory process in the large intestine.

Keywords: mucins, trefoil factor, ulcerative colitis, Crohn’s disease.
Central modulatory role of IL-9 in inflammatory bowel disease influencing barrier function

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Introduction: Interleukin 9 (IL-9) is a pro-inflammatory cytokine mainly produced by T-cells, B-cells and mast cells. Many cytokines have been investigated so far in experimental models of chronic intestinal inflammation, the role of IL-9 is largely unidentified. But high concentrations of IL-9 in the colon tissue during colitis point out the important role of this cytokine.

Methods: For the analysis of the role of IL-9 in chronic intestinal inflammations, IL-9-deficient mice were used in oxazolone-colitis model. Mini-endoscopic analysis has been done to monitor the manifestation of the colitis. The inflamed colon tissue was isolated and histological sections were taken for immunohistofluorescent staining and qPCR analysis. For therapeutically treatment wild-type mice were given specific anti-IL-9 antibody to prevent the emergence of colitis. Bacterial staining (FISH) was done to analyze the barrier reducing effect of IL-9 in mucosa.

Results: In the experimental oxazolone-colitis model the IL-9 KO mice were protected. This became evident in the mini-endoscopic analysis as well as in the HE staining. Immunofluorescence staining shows a decrease of the IL-9-regulating transcription factor PU.1 in the IL-9-deficient mice, indicating the involvement in the IL-9 production. This is consistent with the fact that PU.1 is higher expressed in human biopsies of colitis patients, indicating a pro-inflammatory role of IL-9 in patients. Further analysis of the pro-inflammatory effect of IL-9 showed that the blockage of high IL-9 concentrations with a specific anti-IL-9 antibody in wild-type mice lead to a protection in oxazolone-colitis model. In IL-9 KO mice colon mucosa less bacteria can be found vs. wt mice.

Discussion/Conclusion: Here, we identified a central pathogenic role for the pro-inflammatory cytokine IL-9 in chronic intestinal inflammation. This is based on the fact that IL-9 is increased in inflamed colon tissue and IL-9 KO mice are protected in the experimental oxazolone-colitis model. Furthermore, administration of a blocking anti-IL-9 antibody before the manifestation of colitis has a protective effect. IL-9 seems to have an effect of barrier function in mucosa, because of less bacteria burden in IL-9 KO mice. Thus, IL-9 emerges as a potentially new therapeutic target for inflammatory bowel diseases.
Increased intestinal permeability in patients with ulcerative colitis: Is there a relationship with the disease characteristics?

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Aim: To study the alterations in intestinal permeability (IP) and its relationship with the disease characteristics (extent, severity and endoscopic activity) in patients with active ulcerative colitis (UC).

Methods: Twenty six patients with active UC and 25 healthy subjects consented to participate in the study. The extent and severity of UC were estimated using Montreal classification; the endoscopic activity – using the Mayo scoring system. IP was assessed by the iohexol, which was administrated orally (25 mL, 350 mg/mL) 2 hours after breakfast. Six hours later serum iohexol concentrations (SIC mg/L) were determined by a validated HPLC-UV technique.

Results: Abnormal IP was found in 30.8% of the patients and in 8% of the healthy subjects (p < 0.05). The mean value of SIC (2.49 ± 2.80 mg/L) in the UC patients was significantly higher (p < 0.05) compared to those of healthy controls (1.11 ± 1.10 mg/L). IP alterations were 2-fold more frequently in the patients with extensive UC (pancolitis) (41.7%) than in those with distal and left side UC (21.4%). No relationship was found between IP, assessed by SIC mg/L and UC severity. Significantly higher values of SIC mg/L (p < 0.05) were established in patients with severe endoscopic activity of UC compared to those with mild and moderate activity (3.68 ± 3.18 vs. 0.92 ± 0.69 mg/L).

Conclusions: Increased IP was found in 1/3 of UC patients; more frequently in the cases with pancolitis, and was not related to the disease severity. Serum levels of iohexol can be a reliable disease marker in patients with UC as they reflect the activity of intestinal inflammation in the colon.
Inflammatory bowel disease in the elderly: Later is better than earlier

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**Introduction:** There are enough data confirming a more aggressive course of early-onset inflammatory bowel disease (IBD). This seems to be true particularly in patients with Crohn’s disease (CD) and in a lesser extent in patients with ulcerative colitis (UC). On the other hand data there are very few data considering disease course in patients with late-onset IBD (> 70 years of age). Aim of the study was to evaluate the course of the disease in elderly patients initially diagnosed with IBD.

**Methods:** Data were collected from our IBD database for patients diagnosed with IBD between 2000 up to end of 2012. Seven patients were diagnosed for the first time with IBD, 5 with CD (4 males, 1 female, age range 74–79) and 2 with UC (1 male, 1 female, 76 and 81 years old, respectively). In all patients disease was diagnosed endoscopically and with imaging techniques (CD) and confirmed with histology. Disease severity was mild to moderate according to established criteria of severity (CDAI for CD and Mayo score for UC). From the 5 CD patients 3 had enterocolitis and 2 had colitis, while both UC patients had left-sided disease. All patients were followed-up for a period ranging from 30 up to 74 months.

**Results:** Five out of seven patients (4 with CD and 1 with UC) experienced a favorable clinical and/or hematological and/or endoscopical response with oral and/or topical 5-ASA compounds and remained in remission with oral 5-ASA compounds through-out the follow-up period. Two out of seven patients (1 CD, 1 UC) entered remission after an initial course of oral steroids in a tapered manner and continued to be in remission with maintenance treatment with 5-ASA compounds during all the follow-up period. Treatment regimes were well tolerated from all patients. Follow-up endoscopy (2 CD, 2 UC) revealed significant endoscopic improvement. None of the patients needed a repeated course of steroids, immunosuppressives or use of biological agents. Two out of seven deceased (1 CD [stroke], 1 UC [hip fracture]) years after initial diagnosis and while in remission from diseases unrelated to IBD.

**Discussion/Conclusion:** Even if the number of patients is relatively small, it seems that late-onset (elderly patients) IBD (particularly CD) carries a considerably more benign course compared with early-onset disease in childhood and adolescence.
Case report of intestinal amebiasis complicated by severe bacterial sepsis

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Introduction: During intestinal amebiasis outbreak in Tbilisi, there were observed complications of disease in a variety of forms. The majority of complications were liver abscesses. In rare events, mainly according to the late treatment, were manifested peritonitis, which in part of cases were related to the incidence of intestinal perforations, in other ones – with rupture of abscesses of liver amebiasis. Other bacterial complications were immensely rare, from which we want to describe one clinical case.

Methods: 54-year-old female patient was admitted to our hospital with complaints of fever, diarrhea 4–5 times a day. The stool microscopy investigation revealed E. histolytica cysts. The diagnosis of intestinal amebiasis was verified. Antibiotic therapy was initiated with metronidazole that led to the termination of fever and diarrhea on the 4th day. However, after 3 days fever updated at 39.8° C. WBC – 17.9 mm$^3$. Developed infectious-toxic shock. Was excluded intestinal perforation and peritonitis. The blood culture performed positive for E. coli. With pathogenetic therapy of the shock and corrected antibacterial therapy due to the results of the susceptibility of the isolated bacteria from the blood, the patient’s condition improved and discharged recovered after 10 days of therapy.

Discussion/Conclusion: Infringement of intestinal barrier caused by intestinal amebiasis may create favorable conditions for invasion of commensial microbes of intestines in the blood and develop severe sepsis.
Can you similar to endoscopic and clinical presentation with Crohn's diseases chronic mesenteric ischemia?

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Introduction: Chronic mesenteric ischemia can lead to multiple ulcerations in colon and small bowel. However, these ulcerations' endoscopic imagings may be rarely mimicking Crohn's disease. We present a case whom mesenteric ischemia mimicking Crohn's diseases.

Case report: 57 year-old woman was admitted to emergency department complain with onset abdominal pain and bloody stool. Her history had been cerebrovascular embolic disease and atrial septal defect was repaired by angiographic method. Clopidogrel 75 mg/day had been after stroke. Physical examination was revealed afasia, arterial tension 100/80 mmHg, heart rate 85/min. Abdominal examination detected tenderness upper right quadrant. Defance and rebound were not detected. Laboratory examination was showed anemia (Hg: 9.4 g/dl HCT 29%, WBC 14,200 mm³, thrombocyte 198,000 mm³). Liver, renal and other tests were detected with normal range. Colonoscopic examination was demonstrated multiple ulcers that Crohn mimicking at full length colon. Terminal ileum entubated and showed severe edema and submucosal hemorrhagia and ulcers. Pathological examination was showed chronic ischemic changes that necrosis, hyalinasition. Abdominal computerized angiography was showed occlusion (40–50%) superior mesenteric artery origin and filling defect of inferior mesenteric artery distal branch. We think that chronic mesenteric ischemia. Oral feeding was stopped and antibiotics, IV fluids were initialeted. The patients follow-up all colonoscopic imaging complet recovered.

Discussion: Endoscopic and clinical findings of chronic mesenteric ischemia may be similar to Crohn's disease. Therefore, physicians should be awake the differential diagnosis of Crohn's disease.
The first line of defense: Caspase-8 has a host-protective function through permission of controlled shedding

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Introduction: The intestinal epithelium is equipped with numerous Toll-like receptors (TLR) that play a pivotal role in pathogen recognition, initiating of immune response and cell death activation of infected cells. Beside apoptosis, we have recently discovered necroptosis as a new form of epithelial cell death and a potential pathogenic mechanism driving the development of ileitis in Caspase-8ΔIEC mice and Crohn’s disease patients. However, the triggering factor for necroptosis is still unknown. Therefore, we elucidated whether TLR activation is able to activate epithelial caspase-8 and if lack of caspase-8 switches TLR-induced cell death to necroptosis.

Methods: Injection of Poly(I:C) and LPS into different transgenic mice.

Results: TLR-ligands induced a dramatic villous atrophy and severe destruction of the intestine of Caspase-8ΔIEC mice as compared to control littermates, leading to the death of the former mice within 6 hours. Immunohistochemistry revealed an excessive number of dying epithelial cells with necrotic morphology after TLR-stimulation in Caspase-8ΔIEC mice, but not in Rip3-/-/Caspase-8ΔIEC animals, indicating that this form of cell death is due to Rip3-mediated necroptosis. Moreover, we discovered that Poly(I:C) triggered necroptosis was directly mediated via the TLR3-TRIF pathway, whereas LPS-induced programmed necrosis was prevented in Tnf-R1-/-/Caspase-8ΔIEC mice, indicating the influence of TNF-alpha in this setting. Beyond we could demonstrate that TNF-alpha is produced by gut immune cells and not by intestinal epithelial cells.

Discussion/Conclusion: Our data demonstrate a critical role for caspase-8 in maintaining gut barrier in response to mucosal pathogens through permission of host protective inflammatory shedding and inhibition of necroptosis in infected cells.
The value of neutrophils morphology in predicting infectious episodes in IBD patients

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Introduction: Several infectious intestinal complications appear in the course of the inflammatory bowel disease (IBD), potentially life-threatening and requiring rapid options. A large panel of laboratory biomarkers has been studied in IBD. Earlier therapeutic decision could be taken if a marker could reliably anticipate infections before clinical evidence. The morphology changes of neutrophils could be this marker. Infection leads to increased prominence of azurophilic granules of neutrophils, even in the absence of leukocytosis

Methods: Aim: To assess the value of toxic granulated neutrophils as a marker for predicting abdominal infection in IBD patients.
We examined 106 patients with IBD, with no clinical sign of infection. Peripheral blood samples were obtained from all patients. Automated complete blood cell count for WBC and ANC, and peripheral blood smear preparation was performed. Blood smears were prepared and stained May-Grünwald-Giemsa. The patients with toxic granulations in the neutrophils were closed monitorized for infections, and we noted the time until the first clinical sign of infection.

Results: In 39 patients we noted the presence of neutrophils with toxic granulations. They developed infectious abdominal complications: 26 cases (66%) abscesses, 5 with superimposed infection (12%), 5 developed endotoxemia, 3 (7.6%) had opportunistic infections. The time to infection was 12.2 hours (4–30). WBC: 11.4 x 10⁹/l (2.9 x 10⁹/l to 50 x 10⁹/l). Cultures from local fluid collections or blood were positive in all 39 patients. The sensitivity for predicting a bacterial infection was best for neutrophil toxic granules (79%); ANC (55%), WBC (40%). In combination: high WBC + high ANC: 59%, high WBC + high ANC: 64%, high WBC + high ANC + morphologic changes: 98%.

Discussion/Conclusion: Morphologic changes in neutrophils could be a sensitive and specific marker for predicting acute bacterial infection in patients with IBD. The combination of more parameters helps a prompt therapeutic decision for the benefit of these patients.
Diagnostic pitfalls of dermatological lesions in IBD patients

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Introduction: Major dermatologic manifestations represent an important issue in IBD patients care: Several reports show an incidence of 2–34% in these patients. A large spectrum of skin diseases may occur, from Erythema nodosum (EN) and pyoderma gangrenosum (PG) – the most common, to psoriasis, oral aphthous stomatitis, and Sweet syndrome. The presence of a dermatologist is mandatory in the management team of IBD patients.

Methods: We present a patient with mild Crohn’s disease (CD) and atypical important skin lesions.

Results: Female patient – age 57, 1 year history of CD mild chronic inflammation affecting colon only, well-controlled by mesalazine 3 g/day – presented successive cutaneous lesions disseminated all over the skin, excluding extremities (face, neck, hands, feet) and with bi-phase evolution: first few days looking like chickenpox lesions, last few days like EN. Patient also reported oral herpes lesions in antecedents. Patient accused only but major aesthetic discomfort, without fever, pain or pruritus. Dermatologist indicated short-term corticotherapy, but the lesions continued to appear. In this case, medical team decided biopsy from a lesion. The result showed typical aspects for herpes simplex lesion, which imposed general and topical antiviral therapy with acyclovir instead of prednisone. After two weeks, our patient was dermatological cured.

Discussion/Conclusion: CD induces a dysbalance of human intestinal microbiota with serious health consequences, such as chronic viral exacerbations or transformation. For any dermatological lesions, an integrated medical team is mandatory in order to ensure the diagnosis and management of the disease.
Disturbance of colon microflora in patients with irritated bowel syndrome

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For definition of tactics of complex therapy of irritated bowel syndrome (IBS) and preventive maintenance of further relapses of disease indispensable is the analysis of condition of colon microflora.

The purpose: To learn disturbance of colon microflora at IBS.

Stuff and methods: In 36 patients with IBS the colon microflora was studied. The stuff for research is obtained during fibrocolonoscopy with the indicating of three points of scrapings: colon transversum, descending colon and from a rectum.

Results: The analysis has shown that for IBS the disturbance of microflora takes place, which one shows by increase as conditionally of pathogenic flora (E. coli – 20.1 ± 4.3 10^8), reduction quantity of bifidobacterium (287.3 ± 26.6 10^6), and occurrence in some cases of the pathogenic microorganisms (staphylococcuses – 24.2 ± 2.5 10^3, lactosanegatives esheriy – 16.6 ± 1.2% etc.). It is necessary to mark that the most expressed disturbance of microbial landscape of colon was watched for the catarrhal-erosive form of lesion of slimy colon.

At the analysis of degree of manifestation of dysbacteriosis were established 3 kinds of flow. The insulated phylum of dysbacteriosis is detected in 8 (22.3%) patients, which one was characterized by change quantity of bifidobacterium and had latent flow. At 16 (44.4%) patients the combined phylum of dysbacteriosis of intestine conditioned by availability of staphylococcuses and conditionally of pathogenic flora – lactosanegatives esheriy was established, owing to what had the moderately expressed local form of clinical flow. In 12 (33.3%) – established deployed phylum of dysbacteriosis was watched the expressed clinical flow conditioned by availability of pathogenic microbial association.

Conclusion: Thus, conducted by us the research demonstrates that in 23.3% patients the insulated phylum of dysbacteriosis is watched. However, for 33.3% the deployed phylum of the dysbacteriosis followed with expressed clinical flow owing to availability of microbial association takes place that indicates necessity of application of local and system therapy for a complex of medical measures.
Possible uses of essential phospholipids in patients with inflammatory bowel disease

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Introduction: Pathological process of intestines in patients with inflammatory bowel disease (IBD) leads to increased permeability of intestinal wall, which increases the toxic load to the liver and is one of the risk factors for liver injury. The aim of the study was to evaluate the effectiveness of essential phospholipids (EPL) in the treatment of liver injury and effect on the quality of life, maintaining normal body weight with the background of 5-ASA basic therapy in patients with IBD. Evaluation was performed using inflammatory bowel disease questionnaires (IBDQ).

Methods: The study included 41 patients with ulcerative colitis with mild and moderate severity. Patients were randomized in two groups. The first group included 21, the second 20 patients. All patients were prescribed the basic therapy with 5-ASA, 3–4 grams per day, depending on the degree of disease activity. Patients of the first group were appointed EPL (1800 mg) during the meal for 3 months, as an adjuvant therapy in addition of 5-ASA.

Results:

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Before treatment</th>
<th>After 3 months of treatment</th>
<th>After 6 months of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBDQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 group (n = 21)</td>
<td>137.0 ± 3.4</td>
<td>174.4 ± 4.9*</td>
<td>192.5 ± 5.8*</td>
</tr>
<tr>
<td>2 group (n = 20)</td>
<td>134.9 ± 3.2</td>
<td>150.6 ± 6.1</td>
<td>178.3 ± 7.2*</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 group (n = 21)</td>
<td>19.8 ± 0.42</td>
<td>21.4 ± 0.51</td>
<td>22.4 ± 0.53*</td>
</tr>
<tr>
<td>2 group (n = 20)</td>
<td>20.1 ± 0.45</td>
<td>21.2 ± 0.54</td>
<td>21.7 ± 0.51*</td>
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*Indicators are reliable after treatment compared with indicators before treatment (p < 0.01).

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Patient groups</th>
<th>Healthy (n = 21)</th>
<th>Before treatment</th>
<th>After 3 months</th>
<th>After 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>1 group</td>
<td>0.47 ± 0.05</td>
<td>0.71 ± 0.07*</td>
<td>0.55 ± 0.05*</td>
<td>0.49 ± 0.04</td>
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<tr>
<td></td>
<td>2 group</td>
<td></td>
<td>0.69 ± 0.08*</td>
<td>0.67 ± 0.07</td>
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<tr>
<td>GGTP</td>
<td>1 group</td>
<td>36.98 ± 1.49</td>
<td>61.6 ± 2.44*</td>
<td>48.14 ± 1.48*</td>
<td>32.4 ± 1.06*</td>
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<tr>
<td></td>
<td>2 group</td>
<td></td>
<td>59.8 ± 2.05*</td>
<td>54.22 ± 1.62</td>
<td>53.73 ± 1.42</td>
</tr>
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</table>

*Data are reliable (p < 0.05)

Conclusion: As a result of the patients’ treatment with IBD, including liver damage, EPL reduce manifestations of asthenic, dyspeptic syndromes, and improves patients’ quality of life.
The gene expression of CXCL16, a chemokine and a scavenger receptor, is increased in the colonic biopsies of children with ulcerative colitis

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Introduction: CXCL16 functions both as a chemokine and a scavenger receptor predominantly detected on macrophages. Its increased expression has been found in the inflamed lesions of patients with Crohn’s disease. CXCL16 has also been connected to phagocytosis of bacterial antigens and T helper 1 immune response in experimental colitis. Our aim was to evaluate the expression of CXCL16 and its receptor CXCR6 in the colonic biopsies of children with ulcerative colitis (UC).

Methods: The expressions of CXCL16, CXCR6 and IL-8, an inflammatory marker, in the colonic biopsies of children with active UC (n=19), UC children in clinical and microscopic remission (n = 9) and control children with non-inflamed colon (n = 14) were evaluated by relative quantitative reverse transcription-polymerase chain reaction. Ribosomal protein 18S was used as a housekeeping gene due to its constant expression.

Results: Expression of IL-8 mRNA in colonic biopsies was increased in active UC as compared to controls (p < 0.001) and UC in remission (p = 0.001). The IL-8 mRNA expression was also greater in UC in remission than in controls (p = 0.005). Expression of CXCL16 mRNA was increased in active UC as compared to controls (p = 0.006) but was comparable to UC in remission (p = 0.36). Again, the CXCL16 mRNA expression was greater in UC in remission than in controls (p = 0.033). There was a significant correlation between the gene expressions of IL-8 and CXCL16 (rs = 0.67, p = 0.01). Expression of CXCR6 mRNA was comparable between the study groups.

Discussion/Conclusion: The gene expression of CXCL16 was increased both in active UC and UC in remission in the colonic biopsies of children with UC as compared to control children with non-inflamed colon. The gene expressions of CXCL16 and the inflammatory marker IL-8 correlated to each other. The possible contribution of CXCL16 in the pathogenesis of UC remains to be elucidated.
The case of colitis caused by *Clostridium difficile* and not associated with antibiotic therapy

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**Introduction:** The diarrhea caused by *Clostridium difficile* is usually associated with the use of antibiotics and antineoplastic agents and is commonly seen as a nosocomial infection. However, there is a recent increase in community-acquired cases which are related to the use of antacid drugs. We are describing a case in which *C. difficile*-associated diarrhea developed in a patient who had not been treated previously with either antibiotics, antineoplastic drugs or proton pump inhibitors and H-blockers.

**Methods:** Patient was a male, 56 years old, surgeon by profession, referred to the hospital at the 10th day of his illness with the following complaints: severe, cramping pain in the abdominal area, loose stool 7–8 times a day, fever 39.5° C. Four years ago he was diagnosed with chronic hepatitis C, liver cirrhosis, diabetes mellitus type 2.

The stool microscopy and bacteriology excluded amebiasis, shigellosis, salmonellosis, and escherichiosis. A and B toxins of *C. difficile* were detected in stool. The treatment with 250 mg vancomycin q.i.d. was started and at the 4th day after the treatment initiation, diarrhea, abdominal pain and fever were resolved. On the 5th day after the treatment discontinuation, the diarrhea – loose stool 3–4 times a day and temperature of 37.3° C came back. The treatment was restarted with 125 mg vancomycin q.i.d. for 15 days, after which no relapse was detected.

**Discussion/Conclusion:** The described case is interesting for practicing physicians because *C. difficile*-associated colitis was detected without previous antibiotic therapy on the background of chronic hepatitis and diabetes. As to the *C. difficile* invasion, it could be explained by the fact that the patient works at the hospital.
The role of premorbid gastrointestinal conditions in the development of amebiasis

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Introduction: In 1998–2003, there was an outbreak of intestinal amebiasis, caused by drinking water in Tbilisi, Georgia. More than 500 patients with amebiasis complicated with liver abscess referred to the Antisepsis Center. The analysis of epidemiological data showed that there was practically no evidence of cases in family members of patients, despite of living in the same household, drinking from the same water source and sharing the same sanitary conditions. It led us to the hypothesis that preexisting/premorbid gastrointestinal (GI) conditions could be contributing factors to the development of the amebiasis infection.

Methods: To test this hypothesis, we conducted a retrospective analysis of medical charts of 528 patients with intestinal amebiasis. 72% of patients turned out to have some GI complaints before they developed amebiasis. These complaints included alternating constipation and diarrhea, with predominance of constipation and abdominal distension. Two patients had large intestinal diverticulosis verified by colonoscopy, 12 patients had intestinal polyps of different locations. 28% of patients had no pre-existing GI complaints.

Discussion/Conclusion:
1. Gastrointestinal conditions including chronic constipation, diarrhea, intestinal polyps, and diverticulosis weakens the barrier of the GI tract and increases the likelihood for the development of intestinal amebiasis and its complication with liver abscess.
2. The absence of premorbid disorders of the GI tract does not exclude the possibility of developing intestinal amebiasis.
Increased transepithelial transport of *E. coli* LF82 via the follicle-associated epithelium in ileal Crohn’s disease

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**Introduction:** Postoperative recurrence of Crohn’s disease (CD) remains a major clinical challenge. Luminal bacteria are important triggers of recurrent CD. The earliest signs of CD are apthoid lesions over the follicle-associated epithelium (FAE). The invasive *E. coli* LF82, expressing long polar fimbriae (LPF), has shown to adhere specifically to ileal mucosa of patients with CD. Our aim was to study transepithelial transport of LF82 and the role of LPF in FAE of CD and controls.

**Methods:** Specimens were taken from the terminal ileum from patients undergoing surgery for CD (n = 10) and colonic cancer (n = 11), as non-inflammatory bowel disease (IBD) controls. Regions of FAE were identified and mounted in Ussing chambers for permeability measurements. The paracellular probe $^{51}$Cr-EDTA and live *E. coli* LF82°/LPF were added to the mucosal sides of the tissues. Bacterial passage was measured by fluorimetry and $^{51}$Cr-EDTA passage was measured by gamma counting. In addition, serosal buffers were cultured on agar plates over night.

**Results:** There was a three-fold increase in the passage of LF82°+LPF in CD compared to controls. Moreover, LF82°+LPF significantly increased the passage to $^{51}$Cr-EDTA in FAE of CD, but not in non-IBD controls. The passage of LF82°-LPF was significantly lower than LF82°+LPF in both CD patients and controls. Bacterial culture on agar plates confirmed the fluorimetry results.

**Discussion/Conclusion:** Our findings demonstrate an increased LF82 passage in CD compared to controls, suggesting alterations in the barrier to LF82 in CD. Results further show that LPF plays an important role in facilitating the translocation of LF82 through FAE. Our data present insights into the pathophysiology of CD and might help elucidate and define novel therapeutic targets in CD to prevent recurrence before clinical symptoms and complications.
The impact of iron deficiency without anemia and iron deficiency anemia on quality of life in inflammatory bowel disease: A comparative retrospective study

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Introduction: Iron deficiency (ID) without anemia is common in patients with inflammatory bowel disease (IBD). Although treatment of IDA in IBD patients is strongly recommended in clinical practice, there is still no clear cut consensus in the treatment of ID.

Objective: To analyze and compare the impact of ID and IDA on quality of life (QoL) in IBD patients.

Methods: Medical charts of 156 patients (84 male, 72 female) with IBD (97 with ulcerative colitis, 59 with Crohn’s disease) were retrospectively reviewed for the study between January 2011 and September 2012. The data analyzed included the comparative effects of ID and IDA on QoL in IBD patients. All recruited patients were in remission. The clinical activity of ulcerative colitis and Crohn’s disease was calculated according to Mayo Scoring System and Crohn’s Disease Activity Index (CDAI), respectively. Hemoglobin level below 12 g/dL in women and 13 g/dL in men was accepted as anemia. Ferritin level below 30 µg/L in non-inflammatory conditions and 100 µg/L in inflammatory conditions was evaluated as ID. Disease-specific (UK Inflammatory Bowel Disease Questionnaire UKIBDQ) QoL was measured in patient groups of ID without anemia and IDA. Mann-Whitney U test was used to compare group means. Statistical analyses were conducted using the SPSS (Version 12.0) statistical software program (SPSS, Chicago, IL, USA).

Results: There were 52 patients with low ferritin and 23 patients with normal ferritin levels in ID group without anemia. In comparison, no significant difference with respect to the parameters of disease specific questionnaire was found. Twenty four patients with IDA and 33 patients without anemia were included into the study. The social function was the only parameter that was significantly lower ($p < 0.05$) in patients with anemia compared to patients without anemia.

Discussion/Conclusion: The ID without anemia does not affect the QoL in IBD patients. The parameter of social function is the most sensitive and significant factor to be affected by the presence of anemia in IBD patients.
Analysis of intestinal microbiome of a recovered *Clostridium difficile* patient after stool transplant

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**Introduction:** Infections with drug-resistant *Clostridium difficile* and the subsequent disruption of the intestinal flora by antibiotic treatment, lead to intestinal inflammation and in the majority of severe cases to intoxication and death. Reconstitution of the intestinal flora by fecal transplants is a novel and promising approach to treat such kind of infection. In this study, a patient with a *C. difficile*-associated infection received a fecal transplant from a relative. The patient recovered and shows no symptoms till today. Stool samples of donor and a collection from different time points of the recipient were analyzed for estimation of the intestinal microbial diversity.

**Methods:** DNA was extracted from stool samples and used for library preparation in preparation for Illumina sequencing. Barcoded libraries were generated using the NEBNext DNA sample prep Kit according to manufacturer's instructions. Libraries were sequenced on a Genome Analyzer IIx in a 120 base paired-end read multiplex run. Obtained read sets were assembled using the CLC Genomics Workbench V5. Standard parameters for read trimming and *de novo* assembly were used applying an increased similarity value of 0.9. ORFs of the individual draft genomes were determined by GLIMMER3 and compared by BLASTX against NRPROT. Best Hits were analyzed using the Megan approach.

**Results:** Sequencing of the donor sample resulted in 102,523,070 reads while the sequencing of the patient samples provided 103,715,676 reads (first time after transfer), 62,180,598 reads (second time) and 101,830,352 reads (third time) corresponding to a total contiguous sequence lengths of about 9,300 Mb, 9,853 Mb, 5,907 Mb and 9,674 Mb. Prediction provided 68,887, 69,997, 97,197 and 72,235 orfs with a minimal size of 90 b.

Taxonomical assignment in Megan revealed a complex pattern of the microbiom. No indication of a persistent *C. difficile* infection was obtained for the donor or the recovered patient by this approach.
Discussion/Conclusion: The analysis of microbiota by Illumina sequencing has proved a challenging and complex task. Application of this technology to analyze the composition of the microbiom with respect to pros and cons of different evaluation procedures, data processing and management for future studies will be discussed.
Characterization of the antimicrobial activity of high-mobility group box 2

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Background: Antimicrobial peptides and proteins (AMPs) are important effector molecules of the immune system and can kill or inhibit the growth of bacteria, fungi and virus. The aim of our study was the identification of new biological active AMPs. We could isolate high-mobility group box 2 (HMGB2) from human intestinal tissue and measured antimicrobial activity against *E. coli*.

Methods and results: To characterize and further clarify this new function, we recombinantly expressed HMGB2. By using the radial diffusion assay (RDA) we could detect antibiotic effects against gram-negative and gram-positive bacteria of the normal gut flora. To identify the region of HMGB2 which is crucial for its antimicrobial activity, three peptides which represent three parts of the protein were recombinantly expressed and screened for antimicrobial activity. The results indicated that the two DNA-binding-domains HMG-Box A and B are essential for the antimicrobial effects. In an expression study HMGB2 was found in all analyzed stomach and intestinal sections. In addition, the expression in patients with inflammatory bowel disease was analyzed but no significant differences were determined. However, in some stool and mucus samples from patients with Crohn’s disease and ulcerative colitis HMGB2 was detected by using an immunoassay.

Conclusion: HMGB2 exhibits antimicrobial activity against various commensal bacteria of the normal gut flora and is expressed in all analyzed gastrointestinal tract sections. It is likely that HMGB2 is part of the intestinal barrier and protects, together with other AMPs, the intestine from invading microorganisms.
Bile acids regulate innate barrier functions of colonic epithelial cells

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Intestinal epithelial cells constitute the first line of defence against the entry of toxins and pathogens from the lumen to the body, with several processes contributing to this innate barrier function. For example, fluid secretion flushes pathogens from the lumen, defensin production protects against bacterial invasion, autophagy regulates cell survival and aids in clearing bacteria from infected cells, and restitution repairs the epithelium after injury. Although bile acids are known to be involved in the pathogenesis of IBD, the mechanisms by which they regulate epithelial barrier function are not well understood. Therefore, the aim of this study was to investigate the effects of deoxycholic acid (DCA), the most common colonic bile acid, on epithelial responses that contribute to barrier function.

T84 colonic adenocarcinoma cells were grown on permeable supports and were treated with DCA (50 µM -1 mM) for 1 h to 72 h. Protein expression was investigated by western blotting and mRNA expression by qPCR. Epithelial restitution in polarised T84 cell monolayers was investigated using a wound healing assay. Muscle stripped sections of human colonic mucosa were mounted in Ussing chambers for measurements of ion transport by monitoring changes in short circuit current (Isc) under voltage-clamped conditions. All experiments involving human tissue had the approval of Beaumont Hospital Ethical Committee.

DCA (150 µM, 48 h) attenuated restitution of mechanically wounded T84 cell monolayers by 2.08 ± 0.14 fold compared to untreated controls (n = 3; p < 0.05). Furthermore, DCA (200 µM, 24 h) stimulated autophagy in T84 cells, as indicated by a 1.8 ± 0.9 fold increase in the expression of the autophagy marker, microtubule-associated protein light chain 3 (LC-3 II), when compared to controls (n = 5; p < 0.001). In contrast, the more hydrophilic bile acid, UDCA (50–500 µM) was without effect on LC-3 II expression. DCA also increased expression of mRNA for the constitutively expressed hβD-1 by 7.0 ± 0.9 fold, compared to untreated cells (n = 5; p < 0.001). Levels of inducible hβD-2 mRNA were also significantly upregulated by 4.3 ± 0.4 fold in response to DCA (n = 4; p < 0.01). When added to voltage clamped sections of human colonic tissue, DCA, at concentrations of 200 µM and 500 µM, stimulated Isc responses of 4.0 ± 0.0 µA/cm² and 24.0 ± 0.0 µA/cm² (n = 2). Furthermore, DCA pretreatment attenuated Cl⁻ secretory responses to the Ca²⁺ dependent agonist, carbachol (CCh, 100 µM) from 320 ± 274 µA/cm² to 56 ± 3 µA/cm², n = 2). At concentrations of > 500 µM, DCA rapidly damaged colonic tissues, as demonstrated by a 5.7 ± 0.9 fold increase in transmucosal conductance (n = 2).

Taken together, these data implicate colonic bile acids, such as DCA, as important regulators of intestinal epithelial barrier function and suggest they might be good targets for the development of new approaches to treat IBD.
Reconstructed oral epithelium as model to study probiotic – host – pathogen interactions

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The commensal yeast *Candida albicans* is part of the normal microflora of the oral cavity and can become pathogenic when the host immune system is weak. Several probiotic *Lactobacillus* species are described that exert protective effects on *C. albicans* and other infections in vivo and in vitro. Therefore, we choose *L. rhamnosus* GG (LGG) to investigate the effect of this species on oral *C. albicans* infections.

Using a model system of based on reconstituted human or mouse oral epithelium (RHE) we investigate different aspects of probiotic/host/*Candida* interactions.

Our results indicate a protective role for LGG in our model. *Candida*-infected RHEs pretreated with LGG showed significantly lower levels of lactate dehydrogenase (LDH), used as a marker of cell damage, compared to LDH-levels of untreated RHEs. Protection can also be confirmed by light microscopy where epithelium with co-cultured *Candida* and LGG resemble untreated controls whereas epithelium cultured with *Candida* alone is strongly damaged.

Keratinocytes derived from knock-out mice lacking TLR4 or MyD88 enable us to study the role of these molecules in probiotic/host/pathogen interactions. Preliminary results indicate, that RHEs raised of these knock-out keratinocytes respond much slower to a *C. albicans* infection compared to RHEs raised of wild-type keratinocytes. Finally, the possibility to supplement the RHEs with different immune cells, e.g. PMNs, provides the opportunity to further investigate not only the interaction of the epithelial cells with probiotics and pathogens, but also the role of the immune cells and how they are influenced by probiotics in presence or absence of a pathogen.
C-Jun N-terminal kinases improve gastrointestinal barrier function

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Introduction: The integrity of the gastrointestinal (GI) barrier is major importance to maintain the homeostasis in the gut and to protect the organism from potentially harmful lumen antigens. Impairment of the GI barrier may lead to the development of inflammatory bowel diseases and food allergies. Cell culture experiments have shown that proliferation, differentiation, apoptosis as well as the production of cytokines and antimicrobial peptides in intestinal epithelia cells (IEC) are regulated by c-Jun N-terminal kinases (JNK), a member of the mitogen-activated protein kinase family. Two isoforms of JNK, JNK1 and JNK2, are expressed in IEC.

Methods: Here we used knockout (KO) mice to study the role of JNK in IEC in vivo. Since double KO mice for JNK1 and JNK2 are lethal, we created mice with a conditional JNK1 KO in IEC (JNK1ΔIEC) and crossed these animals with complete JNK2−/− mice in order to generate a complete deletion of JNK in IEC (JNK1ΔIEC/JNK2−/−). We induced colitis with 3% dextran sodium sulfate for 7 days. Animals were daily evaluated to obtain a disease activity index.

Results: Compared to wt animal, disease activity was slightly increased in JNK1ΔIEC mice and more enhanced in JNK2−/−. JNK1ΔIEC/JNK2−/− mice showed the highest disease activity. This data correlated with (i) the histological analysis (ii) increased IEC apoptosis indicated by the presence of cleaved caspase 3, (iii) impaired GI barrier function analyzed by the uptake of FITC-dextran after oral gavage, and (iv) increased inflammatory response measured by the presence of myeloperoxidase as a marker for granulocyte infiltration and the expression of pro-inflammatory cytokines in intestinal tissue.

Discussion/Conclusion: In summary, our data indicate that JNK1 and JNK2 in IEC are elusive during not-pathogenic conditions but improve GI barrier functions in the course of stress responses. Further investigations are required to further elucidate the mechanism how JNK improves GI barrier functions.
Tissue-nonspecific alkaline phosphatase heterozygous mice are protected against DSS experimental colitis due to an increase of T cell response

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Introduction: Alkaline phosphatase (AP) is a membrane-bound metalloenzyme which catalyzes the hydrolysis of phosphomonoesters at alkaline pH. There are four distinct but related alkaline phosphatases: intestinal (IAP), placental and germ cell and the tissue nonspecific form or bone/liver/kidney (TNAP). In colon the isoform mostly expressed is TNAP but IAP is also expressed. We previously demonstrated that in preclinical models of intestinal inflammation there is an increase in AP in colon and ileum. It is described that AP can dephosphorylate LPS in fact IAP-/- mice are more susceptible to DSS colitis.

Methods: We wanted to know the role of TNAP in colitis. We used the model of experimental colitis in mice induced by addition of dextran sulfate sodium (DSS) 1% (v/v) to drinking water for 9 days. There were 4 groups: 2 nontreated (WT-Control and TNAP-Control) and 2 treated with DSS (WT-DSS and TNAP-DSS). The status of the animals was monitored daily by general examination and specifically by means of the Disease Activity Index (DAI) (general appearance, weight loss, diarrhea and hematochezia).

Results: The body weight loss in TNAP-DSS was -9.3 ± 2.3% vs. -19.3 ± 1.9% in WT-DSS mice, and the DAI was also increased in WT-DSS (11.5 ± 1 vs. 7.0 ± 0). Myeloperoxidase activity was lower in TNAP-DSS (22.6 ± 3.9 vs. 49.5 ± 8.1 mU/mg prot). Histologically, TNAP-DSS mice showed lower degree of infiltration and bowel wall thickening. FACS analysis revealed an enrichment of CD4+ and CD8+ lymphocytes in mesenteric lymph nodes of TNAP-DSS mice, along with an increased production of IFN-γ and IL-10 but not IL-17, suggesting a possible involvement of lymphocytes in the differential response. It should be noted that AP activity was comparable in TNAP and control mice.

Discussion/Conclusion: TNAP appears to play a deleterious role in colonic inflammation.
The flavonoid quercetin fails to attenuate experimental ileitis despite epithelium-dependent downregulation of IFN-γ production

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Introduction: The flavonoids quercitrin and rutin are effective in experimental colitis, acting supposedly as quercetin prodrugs. Since quercetin is released at the colonic level by these glycosides, our objective was to test whether quercetin ameliorates experimental ileitis.

Methods: Rat ileal inflammation was induced by the TNBS method. Treatment with quercetin 11.1 mg/kg (or 1% methylcellulose) administered by gavage was started 2 days before ileitis induction. Six days postinduction the animals were sacrificed and the ileum examined for macroscopic and microscopic parameters. In addition, the effect of quercetin on IFN-γ production by rat T cells was assessed in vitro, either directly or in coculture in Transwells with intestinal epithelial cells (IEC18).

Results: TNBS ileal inflammation was characterized by anorexia and damage score markedly elevated above the control group. The administration of quercetin was able to improve ileal alkaline phosphatase activity (74%) and to decrease IFN-γ mRNA expression (89%) as well as IFN-γ production by lymph nodes cells (90%) relative to TNBS. However, quercetin failed to improve body weight, damage score, weight/length ratio or MPO activity, even though administration of this dose of quercetin to normal rats resulted in substantial levels (1000 pmol/g tissue) at the ileal mucosa 3 h after administration. Quercetin was cytotoxic to T cells in vitro at concentrations above 50 µM, and had no effect on IFN-γ release at nontoxic concentrations. Conversely, apically added quercetin in IEC18-T cell cocultures was able to downregulate IFN-γ production by 34% at the concentration of 1 µM.

Discussion/Conclusion: Oral administration of quercetin is essentially ineffective in experimental ileitis despite the capacity of the flavonoid to downregulate T cell IFN-γ production, possibly via an epithelial action.
Expression of mRNA-specific cytokine profiles in the colonic mucosa of patients with IBD

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Introduction: Cytokines have validated roles in the immunopathogenesis of inflammatory bowel disease (IBD) with its two major forms ulcerative colitis (UC) and Crohn’s disease (CD). The aim of this study was to investigated mRNA expression of transcription factor FoxP3, regulatory (IL-10, TGF-\(\beta\) and IL-6), and pro-inflammatory IL-23 and IL-17A cytokines, in paired mucosal samples from patients with IBD.

Methods: We examined mRNA relative quantities of FoxP3, IL-10, TGF-\(\beta\), IL-6, IL-23, and IL-17A in inflamed and adjacent normal colonic mucosa samples from 37 patients with IBD (23 with UC and 14 with CD) and in normal mucosal tissue in 12 persons without IBD by performing qRT-PCR assay.

Results: All investigated genes are upregulated in inflamed mucosa in the following order, according to RQ value: IL-6>FoxP3>TGF-\(\beta\)>IL-23>IL-17A>IL-10. The gene expression of FoxP3 and IL-6 were significantly higher in inflamed mucosal tissue of the IBD patients than the adjacent normal mucosa (p = 0.035, p = 0.03 respectively), borderline significance for IL-10 (p = 0.05), and no significance for IL17A (p = 0.285), IL-23 (p = 0.213), and TGF-\(\beta\) (p = 0.671). We also observed significant differences between higher gene expression of FoxP3 and IL-6 in inflamed tissue in UC (p = 0.011, p = 0.000 respectively) and CD (p = 0.008, p = 0.000 respectively) compare to normal mucosa of persons without IBD and increased TGF-\(\beta\) in CD patients alone (p = 0.041). Moreover, IL-6 and TGF-\(\beta\) were overexpressed (RQ > 10) in non-inflamed mucosa from IBD patients in comparison with normal mucosa from controls.

Discussion/Conclusion: Although exogenous and infectious agents are contribute to triggering the onset of disease, the mucosal immunity mediates the tissue damage through effect of secreted regulatory and effector cytokines. Our results demonstrated differences in the expression of mRNA encoded regulatory and effectors cytokines in IBD. Obtained specific expression profile included IL-6, TGF-\(\beta\) and IL-10 cytokines simultaneously with the transcription factor FoxP3 may represent a transcriptional hallmark for IBD.
Relation between a single nucleotide polymorphism in MORC4, NOD2 variants and outcome after allogeneic stem cell transplantation for hematological malignancies

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Introduction: There is comparable pathophysiology, in the intestine, in inflammatory bowel disease (IBD) and graft-versus-host-disease (GVHD) after allogeneic stem cell transplantation (SCT) for hematological malignancies. Studies have found an association between the three most common single nucleotide polymorphisms (SNPs) in NOD2 (R702W, G908R and 3020insC) and an increased incidence of acute GVHD. We have recently, in a case-control approach, found a genetic association between a marker in MORC4 and Crohn’s disease. We speculated that the genetic pattern seen in IBD might have impact on the emergence of acute GVHD and mortality risk.

Methods: Adult patients (n = 111, median age 49 [IQR 39–57], 64 men) with hematological malignancies that underwent SCT from May 1996 to April 2005 were included. A MORC4 SNP and the three most common SNPs in NOD2 were investigated for genetic associations to the occurrence of acute GVHD and the mortality risk after SCT, using a case-control approach.

Results: Forty patients (27 men) died within 12 months after SCT. Since the marker for MORC4 is located on the X-chromosome, we stratified the patients into two groups, men and women. For men (allelic OR = 4.21, 95% CI 1.31–13.48, p = 0.012), but not for women (allelic OR = 1.53, 95% CI 0.68–3.45, p = 0.304), the association between MORC4 and mortality was significant. Additionally, the Mantel-Haenszel estimate of the common OR for association between the MORC4 marker and mortality was significant (allelic OR = 2.17, 95% CI 1.10–4.29, p = 0.019). On the basis of carrying none or at least one NOD2 mutant allele, a trend to significant association was identified between NOD2 (allelic OR = 4.21, 95% CI 0.90–19.69, p = 0.071) and mortality. Additionally, no significant association was identified between MORC4 or NOD2 and acute GVHD.

Discussion/Conclusion: These findings reinforce a concept where GVHD and IBD share a subset of genetic susceptibility factors, and may lead to a better understanding of GVHD pathogenesis after SCT for hematological malignancy.
Probiotics isolated from Funazushi modulates NK-1R expression in colonic epithelial cells in vitro and attenuated DSS-induced colitis

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Introduction: We recently isolated a novel probiotics strain (Lactobacillus buchneri strain s193) from Japanese "Funazushi" (Japanese old-style sushi; salted and fermented with rice and a crucian carp). There have been some reports about the effects of probiotics isolated from dairy products such as L. gasseri or intestinal microbiota including E. coli on colonic barrier function, but little is unknown about s193 and its mechanism. In this study we investigated whether s193 can modulate the colonic barrier function of epithelial cells using human cell line, Caco2. Moreover, a therapeutic effect of s193 on experimental colitis in mice induced by DSS was also examined.

Methods: Caco2 cells were stimulated with s193, L. gasseri or E. coli for 6 h. In some tests, the effect of LPS was also investigated. mRNA levels of ZO-1 and NK-1R, a substance P receptor, were determined. In vivo study, administration of s193, L. gasseri or E. coli was performed in mice every day during administration of 3% DSS for 7 days. Colonic inflammation was evaluated by H&E staining. mRNA levels of tachykinin and NK-1R in colonic mucosa were determined.

Results: Although mRNA levels of ZO-1 in Caco2 cells were not significantly affected by all bacteria exposure, mRNA levels of NK-1R up-regulated by LPS were significantly decreased by s193 compared with L. gasseri and E. coli. s193 displayed a dramatic anti-inflammatory effect on DSS-induced colitis compared with L. gasseri. Although the increased mRNA levels of NK-1R in the colonic tissue induced by DSS was also significantly attenuated by s193 administration compared with control, the reductions were not observed in E. coli administration.

Discussion/Conclusion: These data suggest that s193 may have anti-inflammatory effect on DSS-colitis not by the promotion of tight junction molecule but rather by reductions of neuropeptides such as substance P and its receptors in colonic epithelial cells.
Changes in the colonic transportome evoked by the total or subtotal absence of microbiota in mice and modulation by bacterial antigens

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Introduction: Animals reared in germ-free (GF) conditions show signs of impaired water homeostasis, as shown by loose stools, hemoconcentration or increased water intake. The aim of this study was to study the expression of transporters and related genes in GF conditions and to assess whether this may be reproduced in a ‘pseudo GF’ model.

Methods: NMRI mice were raised either under germ-free or conventional conditions. Some germ-free animals received a sterile bacterial homogenate (SBH). C57BL/6J mice were administered an antibiotic cocktail (ampicillin, neomycin, vancomycin and metronidazole) in drinking water for 4 weeks to achieve a subtotal removal of the colonic microbiota. Bacterial depletion was assessed by measuring 16S in DNA isolated from feces. Total RNA from colon and caecum was isolated and analyzed by RT-PCR. In addition, a microarray analysis was performed in GF mice by using an Illumina mouseWG-6 v2.0 array platform.

Results: The colon of germ-free mice had a significantly increased expression of endothelin 1, aquaporin 8, DRA, NHE3, the alpha-, beta- and gamma-subunits of the epithelial sodium channel (ENaC), as well as the alpha- and beta-subunits of the sodium/potassium ATPase. This upregulation was partially inhibited by SBH. These results were confirmed in the ‘pseudo GF’ model, in which a significant increase in the colonic expression of endothelin 1, aquaporin 8, the alpha- and beta-subunits of the sodium/potassium ATPase and the alpha- and gamma-subunits of ENaC was observed compared with the control group. In the cecum of these mice, similar changes were noted but DRA and NHE3 were additionally upregulated.

Discussion/Conclusion: The alteration in water homeostasis associated with GF conditions can be induced by subtotal depletion of the microbiota. The changes in the transportome are probably determinant to compensate a basic defect of water absorption.
Plasmoblastic lymphoma presenting as a paravertebral mass in a patient with Crohn’s disease after immunosuppressive therapy

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Introduction: Plasmoblastic lymphoma is an aggressive non-Hodgkin lymphoma classically occurring in individuals infected with HIV. Plasmoblastic lymphoma has a predilection for the oral cavity and jaw. However, recent case reports have shown lymphoma in the stomach, lung, nasal cavity and jejunum in HIV-negative individuals. We report what is to the best our knowledge the second case of plasmablastic lymphoma presenting as a paravertebral mass in a patient with Crohn’s disease after immunosuppressive therapy.

Case presentation: A 47-year-old man with a 27 year history of Crohn’s disease. He has also anchilosan spandylitis and bilateral sacroilitis. His maintenance therapy was azathioprine 100 mg daily. After 12 years beginning azathioprine the patient was presented with complaint of pain on his right and left side of chest, weight loss and paraparetic position his legs. There was no pathology cervicothorasic and lumber vertebra CT. On the thorax CT plevr al effusion bilaterally, some athelectatic changes and edema, graund glass view and septal thickness. MRI showed paravertebral mass compressing spinal cord involving posterior elements T3–T10. The patient had an operation in neurosurgical unit and mass taken out possibly. Plasma cells in atypic morphology were found in the plevr al fluid cytology. Epidural excisional biopsy specimen showed plasmositom, plasmoblastic lymphoma.

Conclusion: We described here uncommon presentation of this relatively rare plasmoblastic lymphoma in the form of spinal cord compression syndrome after immunosuppression with azathioprine treatment for Crohn’s disease.
WNT pathway ligands, Paneth cells and small intestinal Crohn’s disease

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Background: To control a large amount of microbes intestinal epithelia critically depend on antimicrobial peptides (AMPs). Those innate “antibiotics” can be produced ubiquitously or by specific cells. One particularly specialized AMP source is the Paneth cell in the small intestine. The cells most prominent products, the α-defensins HD5 and HD6 are decreased in patients with small intestinal Crohn’s disease (CD). This is linked to different Wnt pathway defects, amongst others a coding variant in LRP6, a Wnt co-receptor, which is associated with an early onset phenotype in patients. We now studied the mucosal expression of different ligands for this receptor, including Wnts, R-Spondins and DKK proteins in patients.

Methods: RNA from 114 biopsies (CD patients and controls) was isolated and transcribed into cDNA. Analyzed products were quantified by real-time PCR and normalized to β-actin. Patients were grouped according to the inflammatory state, mRNA level of different ligands were tested for correlation with IL8 or HD6 via Spearman rank analysis. Expression differences were subject to student t- or Mann-Whitney-tests (depending on distribution).

Results: mRNA expression of some Wnt pathway influencing ligands is significantly reduced in patients, while others are unchanged or increased in certain subgroups. R-Spondin 2, e. g., exhibits diminished mRNA independent of current inflammation which correlates with levels of HD6.

Conclusion and future experiments: Different Wnt signaling impairments are seen in small intestinal CD. These malfunctions affect the transcription factor and receptor level and, as now reported, also include altered mRNA expression of important ligands. A functional genetic Wnt pathway approach including ligands in addition to classical intracellular signaling compounds might elucidate further causative variants in small intestinal CD.

This work was funded by the DFG (SFB685) and the Robert Bosch Foundation
Crohn’s disease with isolated small bowel involvement

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Introduction: Crohn’s disease (CD) is an idiopathic inflammatory bowel disease (IBD) that develops in genetically predisposed people. It can affect any area of the gastrointestinal tract from the mouth to the anus. Isolated small bowel disease is relatively rare and makes the diagnosis very challenging.

Methods: A 16-year-old boy visited our clinic with a history of weight loss and anemia, followed by rectal bleeding and periodic periumbilical pain. Based on the standard clinical and laboratory tests we suspected IBD and performed fibrocolonoscopy and fibrogastroscopy with biopsies, and small bowel follow through. All of them did not support the initial diagnosis. We investigated the value of fecal calprotectin (FC) as a surrogate marker of intestinal inflammation.

Results: FC levels were highly elevated. Searching for a diagnosis we performed a video capsule endoscopy (VCE) and the findings were consistent with CD. Based on the clinical symptoms, laboratory findings and VCE we accepted a diagnosis of CD with isolated small intestinal involvement and started an appropriate medical therapy.

Discussion/Conclusion: One-third of all CD cases involve only the small bowel – the most difficult area to access for diagnostic purposes. VCE is a quite new method that expands the diagnostic capabilities in the small bowel. Measurement of FC levels in patients with clinical suspicion of CD and negative bidirectional endoscopy could be a useful screening tool to select patients with possible isolated small bowel CD who are suitable for VCE.
Fluctuations in butyrate-producing bacteria correlate with the production of pro-inflammatory cytokines in different stages of ulcerative colitis

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Introduction: In IBD, a severe GI gut dysbiosis and pro-inflammatory state exists. Gut bacteria also regulate pathways of T cell differentiation as well as the balance between anti-inflammatory and pro-inflammatory cytokines in IBD patients. Patients with UC and CD exhibit different cytokine profiles, which are TH2 and TH1 driven. We explored this new paradigm in UC patients vs. control individuals and evaluated the imbalance in gut microflora and the immune dysregulation at the same time in order to achieve better treatment outcomes.

Methods: The expression of inflammatory cytokines was quantified by real-time PCR in colon mucosal biopsies from control, mild, severe and remission stages of UC. We employed fluorescent in situ hybridization in combination with flow cytometry, to enumerate the butyrate population targeting 16S rRNA gene. Concentration of major short chain fatty acids (SCFA) namely acetate, n-butyrate, iso-butyrate in fecal samples was measured by GC.

Results: We observed significant decrease in the members of Clostridium coccoides (control 25.69% ± 1.62 vs. severe 9.8 ± 2.4%; p = 0.0001) and C. leptum clusters (control 13.74 ± 1.05 vs. severe 6.2 ± 1.8%; p = 0.0001) in fecal samples of UC patients. We also demonstrated decreased concentration of fecal SCFA especially of n-butyrate (control 24.32 ± 1.86% vs. severe 12.74 ± 2.75 mmol/μl; p = 0.003), iso-butyrate (control 1.70 ± 0.41 vs. severe 0.68 ± 0.24 mmol/μl; p = 0.0441) and acetate (control 39.51 ± 1.76 vs. severe 32.12 ± 2.95 mmol/μl; p = 0.047) in the fecal samples of UC patients. Simultaneous increase in expression of pro-inflammatory cytokines like TNF-α, IFN-γ, IL-17 and IL-23 genes were observed in intestinal epithelial cells during severe stage that reverted back to normal during remission stage.

Conclusion: Intestinal microbiota are crucially linked to the disease condition and may subsequently favor an inadequate immune response. As a further consequence from altered microbial complexity, production or availability of bacteria-derived metabolites, e.g. the short-chain fatty acid butyrate is reduced in active UC.
Antimicrobial peptides defensins as a link between chronic inflammation and ductal adenocarcinoma of the pancreas

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Introduction: Antimicrobial peptides defensins contribute to innate immunity and epithelial cell-regeneration and play a role in carcinogenesis of various inflammation-triggered malignant tumors. In patients with chronic pancreatitis (CP) incidence of ductal adenocarcinoma of the pancreas (PDAC) is elevated. The aim of our study was to analyze the role of defensins in the carcinogenesis PDAC and its suspected association with CP.

Methods: Defensins were detected in human tissue specimen of healthy pancreas, CP, and PDAC by immunohistochemical staining. Expression levels of defensin alpha 1 (DEFA1) were quantified using mass spectrometry (SELDI-TOF). Furthermore, DEFA1 expression in several pancreatic cancer cell lines (Colo 357, T3M4, PANC-1) and in immortalized normal pancreatic duct epithelial cells (HPDE) was studied in vitro, and the effects of pro-inflammatory cytokines on this expression were investigated.

Results: Immunohistochemistry showed accumulation of the defensins alpha 1, 2, 3 and 5 in malignant pancreatic ductal epithelia. In SELDI-TOF analysis expression levels of DEFA1 tended to be higher in CP than in healthy pancreas (p = 0.3), and levels were significantly increased in PDAC (p < 0.001). DEFA1 expression was found in pancreatic cancer cell lines at a significantly higher level than in HPDE (p = 0.01). After incubation of cancer cells with TNF-α, IL-1β, and IFN-γ, a significant decrease of DEFA1 expression in cell lysate was seen (p < 0.001) combined with a significantly elevated amount of the protein in the cell culture supernatant (p < 0.01).

Discussion/Conclusion: Defensins may be a link between chronic inflammation and malignant transformation in the pancreas. Since DEFA1 is highly expressed in PDAC tissue and in pancreatic cancer cell lines it could play a role in carcinogenesis of PDAC triggered by inflammatory stimuli as seen in CP. Further clarification of this role of defensins may help to establish new diagnostic and therapeutic options in PDAC.
The treatment with aminosalicylates – Safety and adverse effects

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**Introduction:** The best therapeutic choice has in view the characteristics of the patient with ulcerative colitis, the treatment guidelines and the patient’s compliance. Controlling the efficiency of the treatment, the adverse effects and adapting future treatment are equally important stages in inflammatory bowel diseases.

**Aim:** We have recorded the adverse effects of the treatment with aminosalicylates in 27 patients with ulcerative colitis. The patients had mild or moderate active disease, localized on the level of the left colon. Treatment with aminosalicylates was the first line therapy, therefore the patients were given 1.5–2 g mesalazine daily. Before the beginning of the treatment, the patients were taken urine samples, hemogram, functional renal and hepatic tests and were tested for hepatitis viral markers as well. The liver and kidney biochemical tests were made monthly for the first three months and then at 3 months intervals.

**Results:** The adverse effects we recorded were headache in 3 (1.1%) patients, epigastric pain in 2 (0.7%) patients and nausea in 3 (1.1%) patients. They did not require the interruption of the treatment and were reversible after the symptomatic treatment. After variable periods of time, 2 patients had arthralgia at the level of the small joints of the hands and myalgia, both of which showed signs of relief after specific treatment. They did not impose the interruption of the routine treatment for ulcerative colitis. One patient had a slight increase in aminotransferases 3 months after the beginning of the treatment. In this particular case, hepatoprotective medication was added and reached the normal level aminotransferases at the test after the end of the therapy with aminosalicylates.

**Conclusions:** The beneficial effects of the treatment with aminosalicylates, in the above-mentioned dosage, were not devoid of important adverse effects, which might lead to the interruption or modification of the ulcerative colitis treatment.
A net is not enough: Reducing environment modulates antibiotic activity of human alpha-defensin 6

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The human intestine harbours large amounts of microorganisms, including commensal and pathogenic microbiota. The fraction of strict anaerobes increases from proximal to distal, reaching 99% of bacterial species in the colon. In the small intestine, microbial metabolism leads to a reducing environment with a low redox potential of -150 mV.

Defensins, characterized by three intramolecular disulfide-bridges, are key effector molecules of innate immunity that protect the host from infectious microbes and shape the composition of microbiota at mucosal surfaces. We could show earlier that a reducing environment as well the oxidoreductase thioredoxin activates human beta-defensin 1 (hBD-1) by reduction of its three disulphide bridges (Schroeder et al., Nature 2011).

For human alpha-defensin 6 (HD-6), which is expressed exclusively in small intestinal Paneth cells, formation of self-assembled nanonets was described recently as a mode of action against Salmonella (Chu et al., Science 2012). Here we show that under reducing conditions HD-6 effectively kills several anaerobic Gram-positive commensal bacteria of the human intestine. The mode of action is not clear yet but pre-incubation of reduced HD-6 with iron or zinc abolishes killing activity, suggesting a role of metal-binding in the mode of action. While we could also observed net-formation of the oxidized HD-6 peptide for Bifidobacterium adolescentis and E. coli this trapping activity was not observed for the reduced peptide.

Consequently, HD-6 seems two have two distinct antimicrobial mechanisms: Under oxidizing conditions the peptide is able to trap – but not to kill – bacteria while under reducing conditions the peptide kills – but does not trap – bacteria. Current studies are underway to investigate the physiological role of this observation.

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Fecal calprotectin as non-invasive marker of intestinal inflammation in children

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Introduction: Data on fecal calprotectin (FC) in pediatric population are still limited. The aim of this study was to assess the role of FC as a non-invasive biomarker of intestinal mucosa inflammation in different pediatric gastrointestinal diseases and to compare the results obtained from the standard ELISA-based method with those obtained from a novel desk-top device.

Methods: 54 patients – 24 boys (44.4%) and 30 girls (55.6%) with median age 11.35 years were included in the study. Patients were divided into 3 groups: 1.) 24 children with inflammatory bowel diseases (IBD), 2.) 15 children with other non-infectious intestinal diseases and 3.) control group. Fecal samples were collected from all participants. FC was assayed by ELISA method and using the “desk-top” Quantum Blue Reader®.

Results:
- The mean concentration of FC in all patients with intestinal mucosa inflammation (group 1+2) was significantly higher than the mean concentration found in the control group (p < 0.01).
- The mean FC concentrations in IBD patients (ulcerative colitis and Crohn’s disease) were significantly higher as compared to patients with other non-infectious intestinal diseases (p < 0.01) and control group (p < 0.01).
- IBD patients that had undergone recent surgery displayed score similar to controls.
- Quantum Blue Reader® calprotectin levels correlated well with results derived from standard ELISA assays (r = 0.74, p < 0.01).

Discussion/Conclusion: FC is a high sensitive but not disease specific marker of intestinal inflammation. FC concentration can be a useful non-invasive screening test in children suspected for IBD. The Quantum Blue® rapid test is a method of choice for fast and reliable determination of FC levels.
Small intestinal bacterial overgrowth in children with gastrointestinal symptoms

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Introduction: Human intestinal microbiota is characterized by the presence of a large population of microorganisms. Intestinal bacteria remain in balance only in physiological conditions. They constitute barrier, protecting us from infections. Qualitative as well as quantitative disturbance of microflora has a negative influence on the process of digestion and absorption. Small intestinal bacterial overgrowth (SIBO) is defined as a quantitative proliferation of nonpathogenic microorganisms in the initial segment of small intestine over $10^5$ organisms in 1 ml of small intestine content ($10^5$ CFU/ml, colony forming units).

Methods: The study consisted of 83 children (43 girls and 40 boys) aged 4–17 years (mean 11 ± 3.56 years) hospitalized due to chronic gastrointestinal symptoms. After excluding organic disorders, hydrogen breath test (HBT) with lactulose (administered per os) was performed among them. Expired air was analyzed using Gastrolyzer (Bedfont).

Results: HBT result was positive among 59 (71%) patients; 32 (74%) girls and 27 (68%) boys. Gastrointestinal symptoms were analyzed. It appeared that abdominal pain was the most common symptom among 47 (54%) children (27 girls and 20 boys). 18 (21%) patients presented additional symptoms that coexisted with abdominal pain. HBT was also positive in all patients with constipation, in 6 out of 8 children with low body mass or weight loss and in 1 patient with gastro-esophageal reflux symptoms.

Discussion/Conclusion: Among all patients with gastrointestinal symptoms 70% presented positive result of HBT. Abdominal pain was the most common symptom among 75% of children with SIBO. Small intestinal bacterial overgrowth should be taken under consideration as a cause of various gastrointestinal symptoms in children.
Stat3 differentially regulates intestinal epithelial cell antimicrobial peptide production in vivo

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Introduction: Recent data clearly suggest that early activation of the transcription factor Stat3 in intestinal epithelial cells (IEC) is important for mucosal integrity in the context of chronic intestinal inflammation and infectious colitis. Using gene targeted mice lacking Stat3 in IEC or overexpressing constitutively active STAT3 in IEC, we analyzed how Stat3 regulates the expression of different antimicrobial factors in the steady state and in Salmonella typhimurium induced infectious colitis.

Methods: Total RNA from colon, cecum and ileum of 10 week old STAT3IEC-Ko, STAT3cIECtg mice or littermate controls was isolated. The levels of selected antimicrobial peptides (Reg3β, Reg3γ, Ang4, sPLA2, Defa3, Defa5) was determined by qPCR. In some experiments streptomycin pretreated control and gene targeted mice were orally infected with S. typhimurium. The intestinal microbiota in these mice was characterized and compared by next generation sequencing.

Results: STAT3 differentially regulates antimicrobial peptide expression in IEC. Whereas basal and S. typhimurium induced expression of Reg3 proteins, and sPLA2 was largely reliant on the activation of STAT3, defensin levels were not altered. In contrast, Ang4 transcripts particularly in the colon were significantly increased in the absence of STAT3 in IEC suggesting that STAT3-dependent pathways negatively regulate Ang4 production. Moreover, STAT3 deficiency resulted in profound changes in the composition of the ileal and cecal microflora both in the steady state and in infectious enteritis.

Discussion/Conclusion: These data clearly suggest that STAT3 plays a central role in the differential regulation/expression of antimicrobial peptide production in IEC and this may be relevant for gut homeostasis and innate immunity in infectious colitis.
Transepithelial transport of *E. coli* LF82 in an *in vitro* follicle-associated epithelium model

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**Introduction:** Crohn’s disease (CD) is associated with increased numbers of adherent invasive *E. coli* (AIEC). A specific strain of AIEC, *E. coli* LF82, has been identified in the ileal mucosa of CD patients. It is considered that LF82 is able to adhere and invade the mucosa due to the expression of long polar fimbiae (LPF), which is required for transport of bacteria through the follicle-associated epithelium (FAE).

The aim of our project was to study mechanisms of transepithelial transport of LF82⁺⁻LPF in an *in vitro* FAE model.

**Methods:** An *in vitro* FAE model was established by co-culturing Caco-2 clone 1 cells and Raji B cells. In this FAE model, the passage of *E. coli* LF82⁺⁻LPF, and the effect of human alpha-defensin 5 (HD-5) was studied and compared with control model constituting of Caco-2 clone 1 cells alone. The passage of bacteria was measured after three hours by fluorimetry, bacteria viability assay and culturing of bacteria on agar plates.

**Results:** Fluorimetry measurements revealed a 9% lower and a 6.5% lower passage of LF82⁺LPF in the control model compared to the co-culture model. Further, the passage of LF82⁻LPF was 6% lower in the control model and 9.2% lower in the co-culture model compared to LF82⁺⁻LPF. After exposure to HD-5, the passage of LF82⁺⁻LPF was decreased; in the control model by 10% and in the co-culture model by 28%. Results were confirmed by enumerating bacteria on agar plates.

**Discussion/Conclusions:** The present findings demonstrate that *E. coli* LF82 translocation is favored in the FAE model compared to control model and that LPF plays a crucial role in the ability of LF82 to pass the barrier. Moreover, we demonstrate that HD-5 is able to decrease the passage of LF82⁺⁻LPF through the barrier in both control and co-culture model.
Ascites and proteinuria caused by systemic amyloidosis in a patient with Crohn’s disease

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Crohn’s disease (CD) is an intractable chronic inflammatory bowel disease that can affect any part of the gastrointestinal tract. Systemic secondary (AA) amyloidosis is associated with 0.9–2.5% of patients with CD. Renal failure due to renal amyloidosis is one of the most common causes of death for patients with CD. Due to a better understanding of the association of secondary amyloidosis to CD, early diagnosis of this complication is becoming more frequent, but its treatment continues to pose a challenge.

A 18-year-old female patient presented with chronic diarrhea to our outpatient clinic on December 2012. On physical examination, the patient had hepatosplenomegaly, ascites, pitting pretibial edema and growth retardation. Laboratory findings revealed anemia (hemoglobin: 8.5 g/dL), elevated inflammatory markers (erythrocyte sedimentation rate: 83 mm/h, C-reactive protein: 45 mg/L), hypoproteinemia (total protein: 2.9 g/dL, albumin 1 g/dL), normal creatinine (0.5 g/dL) and proteinuria (0.95 g/d). Endoscopic examination showed compatible findings with CD of upper (distal to the stomach corpus) and lower gastrointestinal (according to the Vienna classification: A1, L3+L4, B1) system. Biopsy of lesions in both upper and lower gastrointestinal system revealed non-caseified granuloma formation that further confirmed the diagnosis of CD. Thoracoabdominal computed tomography showed mediastinal and para-aortic conglomerated lymph nodes. Endosonography assisted fine needle aspiration biopsy of lymph nodes revealed findings related with chronic inflammatory reaction that was evaluated as the result of sustained lymphangiogenic effect of long-term active CD. The examination of ascitic fluid was compatible with non-portal hypertensive etiology. The suspected underlying mechanism for proteinuria and ascites was systemic amyloidosis and rectal biopsy was performed. AA-type amyloid deposition was detected in the biopsy specimens from rectum. The patients were started on colchicine, angiotensin-converting-enzyme inhibitor for proteinuria and on adalimumab for CD. The patient was scheduled for follow up in outpatient clinic.

In this case, we aimed to show that untreated CD may present with catastrophic systemic amyloidosis as a result of uncontrolled inflammation of CD. Based on literature review, adalimumab was given to the patient for induction and maintenance of remission of CD.
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Inflammatory Bowel Diseases: Microbiota versus the Barrier

June 7 – 8, 2013
Kultur- & Kongresszentrum
Liederhalle Stuttgart
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