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Dealing with our “In-vironment”: New Aspects in IBD Pathogenesis and Therapy

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Falk Symposium 183

DEALING WITH OUR “IN-VIRONMENT”:
NEW ASPECTS IN IBD PATHOGENESIS AND THERAPY

Basel (Switzerland)
May 4 – 5, 2012

Scientific Organization:
G. Rogler, Zurich (Switzerland)
C. Beglinger, Basel (Switzerland)
J.-F. Colombel, Lille (France)
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Session I

Genetic risk factors in IBD:
Impaired control of the “in-vironment”?
Risk genes in Crohn’s disease: What are the most important pathways affected?

Prof. Andre Franke
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Genome-wide association studies (GWAS) have resulted in the identification of hundreds of genetic loci associated with human complex traits. A key finding of GWAS has been the observation that many disease loci are shared between distinct immune-mediated diseases. Meta-analyses of individual CD and UC GWAS, together with replication studies of the top GWAS hits, have identified 71 CD- and 47 UC-associated genetic regions with genome-wide significant evidence for association. Major CD loci include loss of function risk alleles in the \textit{NOD2} and \textit{ATG16L1} genes, as well as genes also demonstrating highly significant association to both CD and UC, notably \textit{IL23R}, \textit{IL12B} and \textit{CARD9}. Altogether, 99 IBD loci have been well-documented, with 28 loci shared between CD and UC. The presentation will briefly summarize these large studies, highlight some of the main disease genes and discuss their known functions. Finally, insights will be given how Next Generation Exome Sequencing has already begun to identify novel diseases candidates and began to enter clinical practice.
The “promise of epigenetics“: Will it deliver new insights?

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Epigenetics in its scientific definition “is the study of all heritable and potentially reversible changes in genome function that do not alter the nucleotide sequence within the DNA”, but might be considered in simpler terms as the regulation of gene expression. These following epigenetic regulatory mechanisms involve all biological processes in health and disease: acetylation; methylation; phosphorylation; sumoylation/ubiquination; miRs or microRNAs.

Acetylation is regulated by acetyltransferases (HATs) and histone deacetylases (HDACs)/sirtuins (SIRTs), mediating that genes are either repressed or actively transcribed.

Methylation involves DNA methyltransferases (DNAMTs) which add a methyl group to the promoters of genes, such as CPG islands or to a given protein.

Sumoylation controls apoptosis by the interplay of sumo proteins with Sumo-degrading enzymes called sentrin proteases (SENPs).

miRs or microRNAs are short RNA sequences comprised of 20–22 nucleotides, bind to complementary mRNA sequences along with argonaute proteins resulting in translational repression and thereby silencing of gene expression.

Our laboratory has over the past decade studied these epigenetic processes in distinct types of cells derived from rheumatic disorders (1–6).

With respect to Crohn’s disease (CD) it was recently shown by Brest et al. that miR-196 is overexpressed in inflammatory intestinal epithelial cells of patients with CD and thereby down regulates the IRGM protective variant (c.313C), but not the risk-associated allele (c.313T). Consequently, the loss of IRGM expression results in the impairment of E. coli autophagy (7). This is a prime example of functional epigenomics.

References:


State-of-the-Art Lecture I

Genes and “in-vironment”: How will our concepts on the pathophysiology of IBD develop in the future?

Claudio Fiocchi
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The notion that genetic, environmental, microbial and immune factors are the building blocks of inflammatory bowel disease (IBD) pathogenesis has been generally accepted as correct by IBD investigators for about three decades. While this notion is currently undisputed, there have been substantial changes in regard to how each of the four pathogenic components is currently perceived, both on its own as well as in the context of the others. No single component is believed to be so biologically powerful to condition on its own merit the full-blown expression of disease, either Crohn’s disease (CD) or ulcerative colitis (UC). This last concept is important because it automatically implies that it is the biological integration of genetic, environmental, microbial and immune factors that allows IBD to become manifest. Still, whether all four pathogenic components acting together represent the sine qua non for patients to develop disease, or the combination of two or three components is sufficient to trigger CD or UC is presently unknown. Thus, it seems obvious that while the players of IBD pathophysiology have been probably identified, much remains to be done in the future to understand how these players come together to induce IBD. Let’s briefly discuss each pathophysiological component on its own and in the context on the others.

Genes are intrinsically susceptible to evolution-dependent variations caused by multiple factors, but such variations occur sporadically in the general population. Therefore, it is impossible to simply attribute the dramatic increase in IBD incidence and prevalence documented in the last 50 years to major changes in the genetic pool of any given ethnic or geographical group. While genes are relatively stable, how they work and their ultimate functional outcome are clearly influenced by agents and events both outside as well as inside the host. Essentially everything that constitutes the surrounding environment can affect gene expression. This reality is daunting considering the endless complexity of the environment, but is even more overwhelming now that we have unquestionable evidence of how drastically and quickly the environment has been modified in the last one-half century of human existence. Changes in gene function can occur through gene-gene interactions (epistasis) or epigenetics. The new field of epistasis is barely been scratched in IBD, but investigators must be made aware of its powerful modulatory influence in disease pathogenesis. While the functional outcome of a gene function is being modified by epistasis, the same gene may also be the target of epigenetic changes. Epigenetics can be viewed as a system of chemical tags that attach to DNA and its associated histone proteins. These tags are retained through cell division to regulate the access and recruitment of proteins that switch genes on and off during development, cell differentiation and disease. For instance, if an external agent causes a DNA modification responsible for a particular phenotypic characteristic in IBD, the effect of that modification will continue to act in the patient and may even be transmitted 20
to the offspring. If indeed gene expression is continuously altered by environmentally conditioned epigenetic changes the goal of understanding how individual genes contribute to IBD pathogenesis may become a more and more distant possibility.

In addition to the external – or exogenous – environment, investigators are becoming increasingly aware of the powerful influences of the internal – or endogenous – environment. The host’s enormous microbial load and their pathogen-associated molecular patterns (PAMPs) can be seen as endogenous, but for purpose of the discussion so far, let’s consider them as exogenous. This leaves the host itself as a potential modifier of gene expression in IBD. Cells act primarily through their surface receptors and secreted products both of which, under physiological circumstances, are tightly regulated, and cells eventually die by apoptosis retaining their internal molecular components. In contrast, under pathological conditions, cells not only express receptors inappropriately and secrete abnormal types and quantities of mediators, but they die by necrosis (necroptosis), releasing a variety of products that, by not being normally seen by the host, trigger an inflammatory response against these damage-associated molecular patterns (DAMPs). For instance, during a severe attack of CD or UC necrotic tissue ulceration is an obvious and documentable event associated with the release of DAMPs in the local microenvironment. These DAMPs must also be considered as part of the “in-vironment”, with obvious pathophysiological implications for IBD.

In regard to the host’s microbes, the intestine, and the colon in particular, carries the largest load of PAMPs in the body, a load that must be recognized and dealt appropriately to maintain health and avoid pathological inflammation. This is in fact what happens in a healthy person, but not so in IBD patients, who apparently recognize the intestinal microbiota inappropriately, because of an altered immune response, an abnormal composition of the microbiota, or both. This abnormal recognition process is believed to be the key mechanism set in motion by the mucosal immune system that so triggers and maintains inflammation in CD and perhaps UC. In both forms of IBD there is a “dysbiosis”, but whether this is the cause or the result of intestinal inflammation is not known at present. Regardless, an ongoing PAMP-induced inflammation could be regarded as an “in-vironmental” response and a component of IBD pathophysiology. To complicate pathophysiology even further there is mounting evidence that DAMP and PAMP signals come together through the utilization of shared innate immune receptors (Toll-like and NOD-like receptors – TLRs and NLRs, respectively) and, in combination, are responsible for what has been recently called “non-resolving inflammation”, the prototypical response of IBD.

Finally, it is known that the immune system is the effector arm of inflammation in IBD. To what degree the genetic variants associated with major immune response pathways, like for instance those of the TNF-α, IL-10, IL-12 and IL-23 pathways, explain IBD pathogenesis is a hot topic under investigation, and answers are being eagerly sought. Discoveries will certainly be made in the near future showing multiple immune gene-gene interactions in IBD, and those that will affect the above cytokine pathways are most likely to result in amplifying or inhibitory effects on the associated inflammatory response.
In summary, when trying to answer the original question of how our concepts on the pathophysiology of IBD will develop in the future, various fundamental points must be taken into consideration:

• We still do not understand well enough each component of IBD pathogenesis, and more work is needed in each area.
• Studies that look at gene function in the context of IBD must be pursued far more aggressively, rather than continue to look for more and more rare gene variants, whose results will be increasingly less rewarding.
• Gene-gene interactions have so fare been pondered purely at the hypothetical and mathematical level, but not at a concrete biological level; functional studies of gene-gene interaction (epistasis) in the context of human or experimental IBD are an absolute priority.
• Given the modulatory power of external environmental factors, gene-environment interaction must obviously be pursued; given their massive number what factors to select for study is a real challenge but at the moment any area, e.g., foods, additives, diets, pollutants, drugs, microbes, etc. is an easily justifiable choice.
• The active investigation of DAMPs in IBD pathogenesis needs to be amplified; DAMPs are also numerous, but any one of them is worth of investigation right now.
• Finally, functional integration of knowledge from all the above processes and pathways must be undertaken using system biology approaches (based on “omes” and “omics”) if a comprehensive understanding of IBD pathophysiology is ever to occur. Whether this will happen in the next decade or will take longer is an exciting challenge that must be confronted as soon as possible.
Session II

IBD: The “in-vironment” out of control?
IBD: A defensin deficiency?

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Inflammatory bowel diseases are characterized by chronic intestinal inflammation at different sites. Data from animal models as well as human patients including gene-association studies suggest that different components of the innate barrier function are primarily defective. Defensins are important effector molecules of this barrier shield and specific mechanisms which compromise their proper function in IBD have been identified. This includes disturbed epithelial stem cell differentiation as well as lack of bacterial recognition. These recent advances support the evolving hypothesis that intestinal bacteria induce inflammation predominantly as a result of a weakened innate mucosal barrier in genetically predisposed individuals. Recent data which form our current understanding of the primary events of disease will be discussed. Together, these findings should result in new therapeutic avenues aimed at restoring antimicrobial barrier function to prevent a bacterial-triggered inflammatory response.
Do bacteria stress the epithelial barrier?

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The intestinal barrier fulfils an impressive task in separating an enormous abundance of microbes of various kinds from the otherwise sterile host. This barrier clearly involves a variety of cell types that are all required for this functional barrier, with the intestinal epithelium and the mucus barrier playing a particularly prominent role due to their anatomical localisation. Moreover, the intestinal epithelium has emerged as a key orchestrator regulating other innate and adaptive immune branches of the mucosa. The endoplasmic reticulum (ER) stress response has emerged as an important regulator of intestinal epithelial cell function, with several IBD risk genes associated with this pathway. One in particular, X-box binding protein-1 (XBP1), has been shown to confer risk to IBD via rare, hypomorphic variants. Modelling of hypomorphic Xbp1 function specifically in the intestinal epithelium of mice resulted in the spontaneous evolution of intestinal inflammation.

A key feature of hypomorphic function of the intestinal epithelium is the effect of Paneth cells, which are functionally impaired and even completely depleted in the absence of a floxed Xbp1 allele. As Paneth cells secrete abundant amounts of antimicrobial peptides, this has obvious major implications to the structural composition of the intestinal microbiota. Moreover, in the presence of unresolved ER stress, the epithelium reacts hyper-inflammatory toward microbial components, such as Toll-like receptor ligands. As goblet cells, which generate the intestinal mucus as a physical barrier between luminal contents and the epithelium, are also functionally impaired in the context of unresolved ER stress, this might further augment the inflammatory responsiveness of the epithelium.

In addition of genetic risk factors of IBD that impact of ER stress pathways, multiple bacterial products are known to affect this key homeostatic pathway at this body surface so heavily exposed to the environment.

In summary, the unfolded protein response is a key homeostatic pathway that integrates multiple inputs from the environment and thereby modulates innate and adaptive immune mechanisms in the intestine.
Inflammatory bowel disease: Dysfunction of autophagy?

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Inflammatory bowel disease (IBD) with its two major entities, Crohn’s disease (CD) and ulcerative colitis (UC), represents a chronic inflammation of the gastrointestinal tract. In general, IBD is marked by a dysregulated immune response to intestinal microbiota coupled with an intestinal epithelial barrier defect. These events are most likely genetically driven and recent studies identified almost 100 susceptibility genes for CD and/or UC.

Interestingly, genes being part of the cellular innate immunity, such as NOD2, ATG16L1, IRGM and LRRK2, are only associated with CD, but not UC, suggesting a stronger involvement of the intestinal microbiota in the pathogenesis of CD than in UC. Besides NOD2, especially the autophagy genes ATG16L1 and IRGM have gained more and more importance, since a number of studies demonstrated their involvement not only in autophagosome formation and bacterial handling, but also in the regulation of antigen presentation, Paneth cell function, inflammasome activity and the unfolded protein response. Of particular interest seems the functional relationship between the intracellular bacteria sensor NOD2 and ATG16L1. NOD2 is necessary to initiate autophagy by recruiting ATG16L1 to sites of bacterial entry at the cytoplasmic membrane. Dendritic cells carrying the CD-associated variants of either NOD2 or ATG16L1 are defective in autophagy, antigen presentation and bacterial handling. Presence of the CD-associated variation within the ATG16L1 gene results in aberrant morphology and dysfunction of Paneth cells resulting in defective expression of antibacterial factors and an upregulation of pro-inflammatory molecules. In line with this observation, loss of ATG16L1 results in elevated and uncontrolled inflammasome activity. All of those events result in prolonged survival of invading pathogens, such as *Salmonella typhimurium* or *adherent-invasive E. coli*, impaired innate immune defence mechanisms against intestinal microbiota and uncontrolled inflammatory responses. Of note, ATG16L1 variant mice develop a more severe colitis in presence of a murine Norovirus, suggesting a role for viral infections during IBD pathogenesis. Comparable effects have also been demonstrated for IRGM. In particular, defective IRGM has been associated with prolonged intracellular survival of invasive bacteria and recent studies suggest an involvement of IRGM in controlling virus-induced autophagy. Of note, a family of microRNAs, namely miR-196, has been shown to regulate IRGM expression and function.

Taken together, it is obvious that autophagy plays a key role for bacterial handling and the control of inflammatory reactions to invading pathogens. Genetically caused dysfunction of autophagy genes, as in CD, clearly promotes the onset of chronic intestinal inflammation.
Helminths and epithelial cells

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The “Hygiene Hypothesis” states that a lack of early childhood exposure to helminthic parasites and some symbiotic microorganisms increases susceptibility to immune mediated diseases. Exclusion of helminths from our environment along with their powerful effects on host immune development may have contributed to the emergence of immune-mediated disease like IBD. Clinical trials suggest that helminths and perhaps their products have therapeutic potential in the management of these conditions. Their mechanisms of action probably involve induction of several independent immune regulatory pathways. At least part of the protection depends on parasite induction of regulatory-type cytokines and interactions with cells of the innate immune system. They also activate Foxp3+ T cells making them highly regulatory. How intestinal helminths communicate through the epithelial barrier to promote these regulatory pathways remains unknown. It could involve production of molecules that interact with host pattern recognition receptors of innate immunity and perhaps through modulation of intestinal flora. Learning the biology of these fascinating organisms could lead to alternations in life style and development of vaccines that modulate host immunity, which in turn may help prevent IBD and serve as new therapies.
Smoking cessation changes the “in-vironment”

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The human intestinal microbiota and alterations in its complex composition have been identified as an important factor in the pathogenesis of various gastrointestinal diseases, such as IBD or irritable bowel syndrome in recent years. Moreover, evidence for a role of the intestinal microbiota in the pathogenesis of obesity and the metabolic syndrome has been accumulating and distinctive shifts in the relative abundance of the major phyla in obese vs. lean humans and mice have been described.

Differences in the microbial composition between both the main subtypes of IBD – Crohn’s disease (CD) and ulcerative colitis (UC) – as well as between IBD and healthy controls have been identified. In IBD there is a known divergent effect of smoking on the course of disease with a clearly detrimental effect in CD, whereas smoking has been shown to be protective in UC with a lower incidence of the disease in smokers and a more severe disease course after smoking cessation.

Smoking cessation is associated with a weight gain of an average of 7–8 kg in about 80% of individuals. Interestingly the data linking this increase of body weight to a parallel increase of caloric intake is conflicting, and some studies (in contrast to what one would generally assume) have observed weight gain after smoking cessation despite a stable or even decreased total caloric intake.

With the spread of culture independent methods such as high-throughput sequencing our understanding of the complexity of intestinal microbiota and its component genes (microbiome) including host genetic factors that influence microbial development after birth has substantially increased. Nevertheless, knowledge on the precise role of environmental factors, such as nutrition, medication use or smoking on the composition of the gut microbiota is sparse.

Thus, our group aimed to investigate in a controlled human study whether smoking cessation alters microbial composition and identified profound microbial shifts on the phylum to genus levels that interestingly showed similarities to those observed in obese humans and mice. These results suggest a potential complex interplay between two important pathogenetic factors – smoking and intestinal microbial composition – in IBD and obesity.
Session III

The epithelial barrier as border to the “in-vironment”
The intestinal epithelium functions as a selective barrier, allowing digestion and absorption of nutrients while simultaneously blocking the permeation of microorganisms and toxins into the body. To achieve this, the epithelium is supplied by continuously cycling stem cells, which must produce an appropriate diversity of cell types to carry out its functions. In inflammatory bowel disease, the epithelium is disrupted by chronic inflammation and ulceration. Our preliminary genetic studies of patients with new onset Crohn’s disease suggests that disrupted function of intestinal stem cells may be predictive of a more severe disease course. We also confirmed previous findings that Paneth cell genes were misexpressed in patients with Crohn’s disease. In separate studies, we have found that Paneth cells supply essential niche signals to support intestinal stem cells, in particular Wnt ligands. Intestinal stem cells from Atoh1-mutant mice, which are unable to produce Paneth cells, were unable to grow in an in vitro stem cell culture unless supplied with exogenous Wnt ligand. Atoh1-mutant mice had subtle dysregulation of intestinal stem cells in the absence of Niche signals. However, these mice were exquisitely sensitive to chemically induced colitis and colitis-associated cancer. Thus, while the intestinal could adapt to significant alterations of the stem cell niche (absence of Paneth cells), this created a fragile state of hypersensitivity to additional insult. We suggest that this may be a model for disease progression in patients with inflammatory bowel disease, in whom an abnormal stem cell niche may predispose these individuals to disease.
Protein C pathway is one of the major systems that bridges inflammation and coagulation. The protein C (PC) pathway is a well-characterized anti-coagulant system. Produced mainly by the liver as a zymogen, PC is activated on the vascular endothelial cell surface by thrombin-thrombomodulin complex. Once activated, PC inactivates two important cofactors of the coagulation cascade, factors Va and VIIIa which are crucial for thrombin generation. For many years this pathway has been studied for the clotting process, but only recently great progress has been made in understanding other functions of the PC system. Indeed, many evidences demonstrate that this pathway exerts several activities not only involved in the coagulative process but also in inflammation, cell proliferation, apoptosis, stabilization of endothelial barrier and fibrinolysis. A recent study has shed light on a new role of the Protein C system in controlling intestinal permeability function by regulating tight junction molecules and promoting mucosal healing. This presentation highlights these recent insights regarding the complex scenario of the pathogenesis of inflammatory bowel disease.
PPARγ maintains antimicrobial immunity in the colon

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Novel therapeutic principles are urgently needed to cure inflammatory bowel diseases (IBD), including Crohn’s disease and ulcerative colitis. The nuclear receptor peroxisome proliferator-activated receptor-gamma (PPARγ) is essential for intestinal homeostasis in response to both dietary- and microbiota-derived signals. More recently, PPARγ was identified to function as an antimicrobial factor by maintaining constitutive epithelial expression of a subset of beta-defensins in the colon, which include mDefB10 in mice and DEFB1 in humans. Colonic mucosa of PPARγ-mutant animals shows defective killing of several major components of the intestinal microbiota which include Candida albicans, Bacteroides fragilis, Enterococcus faecalis and Escherichia coli. Neutralization of the colicidal activity by using an anti-mDefB10 blocking antibody was effective in a PPARγ-dependent manner. More importantly, a functional promoter variant that is required for DEFB1 expression confers strong protection against Crohn’s colitis and ileocolitis, providing an explanation why certain PPARγ-targeting drug, such as 5-ASA, is efficient in ulcerative colitis but not in CD. These findings support the development of PPARγ-targeting therapeutic and/or nutritional approaches to prevent colonic inflammation by restoring antimicrobial immunity in CD.
Session IV

Environment and “in-vironment”
Why and where to look in the environment in regards to IBD etiology

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The emergence of IBD among the children of immigrants raised in the developed world has pointed to factors in the environment that led the developed world to foster the relative high rates of IBD evident in the past 50 years. The emergence of IBD in the developing world over the past 20 years has led to several considerations as to what has evolved in these developing environs that mirror what has existed in the developed world for some time. The obvious candidate considerations include changing diet, increasing use of western medications including antibiotics and possibly exogenous infections, especially those that may cross international boundaries. The obvious western dietary culprits could be higher fatty food, greater red meat intake and lower fiber intake; or even other food additives in packaged foods. It is unclear whether dietary factors would trigger the intestinal immune system directly or through changing the gut microbiome. Antibiotic use has increased in the developing world and while these drugs may obviously impact on the gut microbiome, the timing of antibiotic administration may be particularly important. It has been shown that children diagnosed with IBD are more likely to have used antibiotics under the age of one than healthy matched controls. Smoking has long been considered an important environmental risk factor since persons with Crohn’s disease are more likely to be smokers than persons without IBD. Further, smokers with Crohn’s disease have a worse course of their disease than nonsmokers. However, among the countries with the highest smoking rates are those with the lowest rates of Crohn’s disease and countries such as Canada and in Scandinavia have among the lowest rates of smokers and highest rates of Crohn’s disease. Hence, it is possible that smoking modulates disease once it appears rather than being causative. Finally, stress has emerged as an important factor in modulating disease. Perhaps the stress of globalization and a modern lifestyle has brought with it an altered gut immune response and the emergence of IBD in the developing world.
Does our food (environment) change our gut microbiome (“in-vironment”): Potential role for IBD?

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Shortly after the publication of the first human genome draft in 2001, it became obvious that a complete understanding of the human biology would only be achieved by combining the analysis of both the host and its surrounding environment. The human gastrointestinal tract (GIT) hosts more than 100 trillion bacteria and archaea which together make up the gut microbiota. The human host provides a nutrient-rich environment while the microbiota provides indispensable functions that humans cannot exert themselves, such as the production of some vitamins, digestion of complex polysaccharides and the shaping of immunological environment of the GIT. 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 ten years ago, the concept of dysbiosis or unbalanced composition of the intestinal microbiota, was introduced in the IBD research field.

However, since most of the bacteria inhabiting our gut are not cultivable to date, until recently little was known about their individual functions. Metagenomics, defined as the analysis of the collective genomes that are present in a defined environment or ecosystem, gives insight into these specific functions. The first extensive catalogue of microbial genes from the human gut described the large variety of traits coded by the intestinal microbiota and an overall metagenome per individual outnumbering the size of the human genome by a factor of 150. The adult-type microbiome was commonly enriched in gene families suggesting a host-microbiota co-evolution towards functionalities favoring energy harvest from diet and bacterial competition.

Recently, 3 distinct ‘types’ of gut composition within the human population have been highlighted. These 3 distinct “Enterotypes” are characterized by the dominant genera (Bacteroides, Prevotella and Ruminococcus) and their co-occurring phylogenetic groups. While the impact of a specific nutrition (probiotics, prebiotics) on specific bacterial populations has been previously described, a positive correlation was observed between habitual diet and enterotypes. Protein and animal fat uptake was linked to the Bacteroides-dominated enterotype while carbohydrate rich diet was associated with the Prevotella-dominated enterotype. Short term diet change and 10 days dietary intervention affected the bacterial species composition but not the enterotype distribution. This recent discovery that the human population harbors at least 3 distinct ‘types’ of gut microbiota composition and that the belonging to one or the other enterotype might be modulated by food opens up new perspectives in the fields of IBD, nutrition and therapeutic strategies.
DAMP’s (danger-associated molecular patterns) and IBD: Is there a connection?

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The innate immune system is of utmost importance for maintaining the local tissue homeostasis in the intestinal mucosa. It must recognize and rapidly respond to microbial antigens and danger signals, thus, providing a first line of host defense. This is primarily accomplished through an array of pattern recognition receptors (PRR’s) that are located in distinct (sub)cellular compartments and bind Pathogen-, and Danger-Associated Molecular Patterns (PAMP’s, and DAMP’s, respectively).

The impact of PAMP’s, in particular, of NOD2/CARD15, which represents an intracellular receptor for muramyl dipeptides, i.e. a component of the bacterial cell wall, in the pathogenesis of Crohn’s disease has been widely established in a subgroup of patients. The involvement of DAMP’s in the pathogenesis of IBD, however, is much less established so far. DAMP’s represent non-pathogen derived triggers of receptors that may also lead to enhanced inflammatory responses. Examples include heat-shock proteins, and the transcription factor HMGB1, which can also become secreted (monocytes and activated macrophages), or released (from necrotic cells), to also exert pro-inflammatory effects. Triggers of the inflammasomes, like crystalline structures (e.g. monosodium urate, cholesterol) or ATP (binding to purinergic receptors) constitute another important group of non-pathogen related mediators of inflammation. Intriguingly, the presence, or absence, of intact inflammasomes (that eventually mediate the processing of precursor cytokines of the IL1 family, like IL1β, IL18, and IL33) also affects the composition of the intestinal microbiota, which in turn may affect the course of intestinal inflammation. Although the ligand(s) for the triggering receptor expressed on myeloid cells (TREM)-1 have not been fully identified yet, circumstantial evidence indicates that danger-associated ligands are the inducers of the TREM1 mediated, excessive induction of pro-inflammatory events, observed in patients with active IBD.

The impact of selected DAMP’s in the pathogenesis of IBD and in experimental colitis in mouse strains will be presented and discussed.
Microparticles and their impact on intestinal immunity

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Microparticles are small (< 1 μm), non-biological particles that are used in food as anti-
caking agents or food colorants. The most commonly ingested compounds are
aluminium silicate and titanium dioxide (TiO₂), the latter being a white pigment used in
toothpaste or sugar toppings. The increasing abundance of microparticles in western
diet raises the question of potential risks associated with gastrointestinal diseases such
as Crohn’s disease (CD). Accumulation of particles has been shown in cells of Peyer’s
patches, but it is not clear whether this also has pathological effects.
NALP3 is a member of the intracellular pattern recognition receptor family and it is part
of the inflammasome, a multiprotein complex containing caspase-1 which activates pro-
inflammatory cytokines IL-1β and IL-18. With regard to recent findings identifying small
particles such as asbestos and monosodium urate as NALP3 activators, TiO₂ may be
another potential target for inflammasome studies.

We found that macrophage like cells readily take up TiO₂ after 6 h. Incubation of cells
with 5 and 20 μg/ml TiO₂ for 6 and 24 h resulted in assembly of NALP3 with caspase-1.
This inflammasome assembly was correlated with secretion of IL-1β, as detected by
western blot and ELISA. In intestinal epithelial cells, TiO₂ also was found to be taken up.
This was confirmed by electron microscopy and elemental analysis, which recognized
intracellular particles to contain almost exclusively titanium. Counting of particles
localized intracellularly revealed a dose-dependent increase of TiO₂-positive cells. In
medium of the basolateral compartment, secretion of IL-18 was measured, also in a
dose-dependent manner. Furthermore, we found increased serum levels of titanium in
patients with active ulcerative colitis

This points to the fact that in humans with a leaky barrier (such as IBD patients) titanium
dioxide microparticles may be taken up by macrophages and intestinal epithelial cells,
may activate the inflammasome and induce IL-1β and IL-18 secretion. This may
aggravate inflammation. TiO₂ is ingested on a daily basis and may have an influence on
gut immunity.
State-of-the-Art Lecture II

IBD and environment: What have we learned in the last 20 years?

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Since the mid 1990’s, an explosion in microbiome, gnotobiotic, genetic and immunologic
techniques has resulted in a dramatic evolution of our understanding of the
pathogenesis of IBD and laid the foundation for a paradigm shift toward individualized
approaches to treatment and ultimate prevention and cures of Crohn’s disease and
ulcerative colitis. These insights have resulted in an etiologic shift from an emphasis on
autoimmune processes and microbial pathogens to our current belief that IBD is the
result of a dysregulated, chronic immune response to a subset of commensal bacterial
species in a genetically susceptible host in which environmental triggers initiate disease
onset and/or flares. Our knowledge of enteric bacteria has exploded from identification
of 200–300 cultivable species to 2000–3000 species detected by molecular sequencing
of 16s ribosomal DNA. This bacterial load outnumbers our human cells 10:1. Functional
analyses of the complex microbiota, which includes not only bacteria but fungi and
viruses, has been dramatically augmented by metagenomic, proteomic and metabo-
lomic technologies, so that we can begin to dissect the structure and function of the
microbial genes that outnumber our human genes by at least 100-fold. These analyses
have led to the concept that a dysbiosis (abnormal balance of beneficial/detrimental
bacterial species) and functionally altered commensals contribute to disease patho-
genesis. Widespread use of gnotobiotic rodents have provided functional evidence that
commensal bacteria provide the constant antigenic drive for dysregulated immune
responses in susceptible hosts, that defined subsets of normal bacteria have both
aggressive and protective functions, and microbial triggers may interact with genetic
susceptibility. These observations lay the foundation for selective therapeutic manipu-
lation of the microbiome. Advances in molecular genetics have permitted an evolution
from twin and familial phenotypic observations to the identification of > 160 IBD risk
alleles that can be functionally characterized as mediating mucosal barrier function,
immunoregulation or bacterial killing. An explosion in immunologic understanding has
revealed functionally different T cell maturation subsets that exhibit plasticity between
functional groups, mechanisms of innate immune signaling in response to microbial
ligands through various receptors, and the realization that discrete microbial agents and
their components/secreted products can activate specific immunologic pathways,
including TH17, T reg and TR1 cells.

Together, these dramatic basic science advances have resulted in a fundamental
change in our therapeutic arsenal and lay the foundation for individualized prognosis
and treatment. Ultimate prevention and cures will be the result of eliminating environ-
mental triggers and manipulating environmental contributing factors, such as diet,
correcting dysbiosis by selective elimination of detrimental and augmentation of
protective microbial species and their metabolic activities as well as correcting
underlying genetic abnormalities. With the rapid pace of current research, it is realistic to
believe that these goals can be accomplished within the next decade for at least some
groups of IBD patients.
Session V

Treatment decisions made easy:
Do we have the disease markers we need?
Circulating antibodies against bacterial wall products: Are there arguments for early immunosuppression?

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The disease course of IBD patients is highly heterogeneous. The majority of subjects with inflammatory bowel disease (IBD) can experience complications at any time during the disease course, such as stricture or fistula formation, as well as need for hospitalizations or surgery. Early immunosuppressive therapies with anti-TNF agents with or without azathioprine have been shown to achieve higher rates of mucosal healing and corticosteroid-free clinical remission. In addition, data is accumulating regarding the potential positive influence of early immunosuppressive therapies on the natural history of IBD, namely a reduced number of hospitalizations and surgeries.

As early immunosuppressive therapy is accompanied by questions about long-term safety and higher costs, the desire for markers that are able to stratify IBD patients is increasing. If tools are available to predict response to therapy, disease complications as well as need for surgery or hospitalizations, one could identify IBD patients at risk that might benefit from more intense immunosuppression. Validated serum markers, determined early in the disease course, but also during disease, could then trigger modifications of the therapeutic strategy.

Until recently our attempts to predict disease phenotype were mainly based on clinical characteristics. Circulating antibodies against bacterial wall products, such as anti-Saccharomyces cervisiae (ASCA), anti-Pseudomonas associated sequence I2 (anti-I2), anti-outer membran porin C of E. coli (OmpC) and antibodies against the bacterial flagellin cBir1 have been investigated for diagnosis of IBD and disease stratification. More recently antimicrobial antibodies against glycan epitopes, such as anti-mannobioside carbohydrate antibodies (AMCA), anti-laminaribioside carbohydrate antibodies (ALCA), anti-chitobioside carbohydrate antibodies (ACCA), anti-laminarin carbohydrate antibodies (Anti-L) and anti-chitin carbohydrate antibodies (Anti-C) have been described. These markers are linked to Crohn’s disease (CD), are associated with genetic polymorphisms, such as NOD2, and are linked to and possibly predictive of complicated CD behavior and CD-related surgery. No association of these antibodies has been found with assessment of disease activity, tissue healing or prediction of response to therapy.

Serologic antimicrobial antibodies are promising tools for the identification of CD patients, who are at risk for complicated disease courses. This predictive ability might be enhanced by the addition of genetic markers. However, no information is available, if clinical decision-making based on a patient’s antimicrobial antibody profile can alter the natural history of these CD patients.

To truly improve daily clinical practice further research needs to be focused on corroborating the predictive capabilities of these markers. Foremost serologic antimicrobial antibodies need to be incorporated into clinical therapeutic trials to assess their role in identifying patients that may benefit from early immunosuppressive therapy.
Clinical risk factors for complicated disease: How reliable are they?

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Crohn's disease is a chronic, progressive, destructive disease. Complicated disease can be defined as the presence of bowel damage (stricture, abscess, fistula) and/or the need for surgery. Natural history studies provide invaluable data on the disease course. In population-based cohorts, half of all patients had experienced an intestinal complication within 20 years after diagnosis and half of the patients required surgery within 10 years after diagnosis. In Olmsted County, factors associated with development of complications were the presence of ileal involvement and perianal disease. Non-colonic disease extent, current smoking, male gender, penetrating disease behavior, and early steroid use were significantly associated with major abdominal surgery. Overall, using various definitions of complicated disease, the predictors of a worse outcome in Crohn's disease were: extensive small bowel, severe upper gastrointestinal disease, rectal disease, perianal lesions, early stricturing/penetrating disease, smoking and young age at diagnosis.

Aggressive ulcerative colitis was recently defined as disease that is associated with a high relapse rate, need for surgery, development of colon cancer, or the presence of extraintestinal manifestations. About one-tenth of patients still require colectomy for ulcerative colitis at 5 years in the era of biologics. An average of 1.6% of patients with ulcerative colitis was diagnosed with colorectal cancer during 14 years of follow-up in population-based cohorts. A younger age at diagnosis and pancolitis were associated with a worse outcome in ulcerative colitis.

The identification of clinical risk factors for complicated disease may be used in future disease-modification trials.
Treatment decision based on biomarkers

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Inflammatory bowel disease is a chronic, recurrent disorder of the gastrointestinal tract, including Crohn’s disease (CD) and ulcerative colitis (UC). Signs and symptoms of acute inflammation vary in terms of localisation and severity, thus making decisions about examinations and therapy management is very difficult. Many tools for clinical assessment of the severity and extend of IBD disease activity have been established, though all having their limitations.

Activity indices such as Crohn’s Disease Activity Index (CDAI) or the Mayo Score, assessing a combination of symptoms, laboratory and examination findings to quantify IBD activity, have been validated in clinical trials [1–3]. Collection of data for these activity indices is tedious and some parameters strongly depend on subjective patient’s symptoms; thus, theses indices are rarely used in daily clinical practice. Laboratory parameters like CRP, blood leukocytes or ESR have a low sensitivity and specificity for bowel inflammation and the correlation with symptoms and disease activity indexes is poor. Endoscopy with mucosal biopsies is the gold standard for the evaluation of extent and severity of disease activity. However, endoscopy is invasive, expensive, not always available and often not very well accepted by the patient. To overcome these limitations a simple, inexpensive, well tolerated and accurate tool is needed to monitor activity of intestinal inflammation in IBD patients.

Monitoring response to treatment
The estimation of treatment response in IBD has largely been based on symptoms, clinical scores and serum markers of inflammation. Data on the value of faecal calprotectin in this setting has long been scarce and evidence has started to grow only recently. In one study patients were treated with 5-aminosalicylic acid (5-ASA) or a combination of 5-ASA, prednisone, or azathioprine. After 8 week, 82% of patients had normal endoscopy and normalization of calprotectin levels were 100% predictive for complete response to treatment. The response of CD patients to anti-TNF agents was investigated in another study In fifteen patients of a previously published cross-sectional study who were considered in need of anti-TNF treatment for acute flare (N = 6), chronic active disease (N = 6) and rapid reoccurrence of the disease postoperatively (N = 3), colonoscopy was performed at baseline and 12 weeks after induction treatment with infliximab in 14 patients and adalimumab subcutaneously in 1 patient. The endoscopic post-treatment activity correlated well with faecal calprotectin values (R = 0.831). In patients with acute severe ulcerative colitis, the value of faecal calprotectin to predict colectomy and to predict corticosteroid or infliximab non-response to treatment was analyzed. Calprotectin levels were higher only in patients requiring colectomy (P = 0.04), but not in corticosteroid (P = 0.08) and infliximab non-responders (P = 0.06). In two studies with paediatric patients with UC, the Paediatric UC Activity Index more accurately predicted treatment response and long-term outcome than faecal calprotectin.
Stool calprotectin levels as surrogate marker of mucosal healing in IBD patients
High faecal calprotectin in patients in remission may represent a stage of enhanced or active mucosal inflammation, which progresses to cause an eventual clinical relapse of the disease, especially in ulcerative colitis. A single calprotectin level had a high predictive value for clinical relapse in one study. Whether calprotectin can be used as sensitive marker for mucosal healing in IBD patients still needs to be proven. Nevertheless, the evidence in this field is growing.

Prediction of relapse in Crohn's disease and ulcerative colitis patients
Recent evidence supports a role for faecal calprotectin in the prediction of inflammatory bowel disease relapses, both in patients with ulcerative colitis and Crohn's disease. IBD patients in clinical remission were studied in a prospective multicenter study with calprotectin and lactoferrin determinations. After a follow-up of 12 months, 16% of patients had relapsed. Patients with a basal calprotectin > 150 mg/l had a 30% risk of relapse, compared to a risk of 7% in patients with calprotectin < 150 mg/l (p < 0.001). Calprotectin concentrations in patients who suffered a relapse were higher than in nonrelapsing patients. High faecal calprotectin levels were associated with clinical relapse in a Kaplan-Meier survival analysis. The authors concluded that faecal calprotectin determination may be useful in predicting impending clinical relapse in both CD and UC patients. For lactoferrin, the corresponding rates of relapse were 25% and 10%, respectively. The sensitivity and specificity of calprotectin to predict relapse were 69% and 69%, respectively. Faecal calprotectin is dramatically increased in severe UC, raising the possibility to be a good predictor for response to first- or second-line medical therapy. However, further studies are needed before any recommendations can be given.

Conclusions
At the present time, measuring faecal calprotectin is a very promising method to monitor and improve management and disease outcome of IBD. There is growing evidence not only that calprotectin levels in patients with present IBD diagnosis are predictive of flares, but that dynamic changes in the calprotectin levels can be used as indication for therapeutic interventions. At the current evidence level it is recommended to measure stool calprotectin concentrations in special clinical situations, such as unspecific and hard-to-interpret gastrointestinal symptoms, to confirm complete mucosal healing and deep remission of IBD and for monitoring of response to a therapeutic intervention.

References:


Intestinal absorption and vitamin levels: Is there need for a new focus?

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Vitamins are micronutrient chemical compounds that cannot be synthesized by an organism and that are essential for life processes. They act as required intermediaries, cofactors, or coenzymes in many processes of normal metabolism or may even have anti-inflammatory effects. In inflammatory bowel disease (IBD) vitamin deficiency is often due to malnutrition and a consecutive anemia. Vitamin B₁₂ and folic acid supplements may be necessary in IBD patients, especially in Crohn’s disease patients or in patients with resection of the terminal ileum and during therapy with sulfasalazine since this inhibits absorption of vitamin B₁₂. Patients with high or continuous activity of Crohn's disease and a frequent therapy with steroids have an increased risk of vitamin D and lower bone density. Vitamin D and bone density should be regularly controlled in these patients and vitamin D should be supplemented. A recent trial even reported a trend to reduce the risk of relapses in CD patients treated with vitamin D.

For other vitamin deficiencies in IBD patients such as vitamin A, B₁, B₂, Niacine, B₆, C, E, and K only limited studies and case reports exist and are summarized in this review. Regular nutritional monitoring in IBD patients is warranted and needs special attention of treating physicians and dieticians.
Session VI

Changing the “in-vironment” for therapy
Specific probiotics or “fecal transplantation”? 

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The overwhelming number of bacteria and the diversity of the enteric flora has 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 diatary supplements such as fibres 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. Certainly, a most physiological, though not selective technique is heterologous transplantation of complete stools.

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. For fecal transplantation the safety issue is quite unclear. Today, casuistic experiences on some hundreads of patients are available but nearly no scientific indepth results. In addition, the health conditions required of the stool donors are not standardised.

Comparing the microbiological and immunological effects. There are also wide differences in knowledge. Only some specific bacteria and one combination has been investigated sufficiently.

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). Therapy of IBS has been widely studied with diets, prebiotics and specific as well as less defined probiotics. Altogether efficacy results are very promising at least for some forms of IBS. Specific and well defined probiotics have shown convincing therapeutic effects in ulcerative colitis and pouchitis but have failed in Crohn’s disease. Fecal transplantation seems to be very effective for CDI while prebiotics and probiotics have not been sufficiently studied as yet. Those 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, bacteriotherapy of specific probiotica and fecal transplantation offers individual approaches to different intestinal diseases. In some diseases good evidence of therapeutic efficacy is existing but altogether bacteriotherapy needs far more research work for final statements.
Mucosal protection by phosphatidylcholine

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Despite the enormous concentration of commensal microbes in stool, the mucosa remains intact and inflammation, erosions or ulcerations are not observed under physiological conditions. This is due to the nature of the mucosal barrier. Defensive mechanisms prohibit the invasion of bacteria and the mucosa associated immune system remains quiescent. Only in case bacteria invade the mucosa, the immune system is rapidly activated to prevent (as last stop) systemic translocation and sepsis. The mucosal barrier consists of the densely packed mucosal cell layer connected by tight junctions. The most important component of the barrier, however, is the mucus layer as first line of defense.

It is organized as a hydrated polymeric gel. Within the tightly packed lower layer, phosphatidylcholine (PC) with its positively charged head group is highly enriched (> 90% of the phospholipids present) and most likely associated to the strongly negatively charged mucins serving as mucus scaffold. The PC is arranged in lamellar structures, the “surfactant like particles (SLP)” which establish a hydrophobic surface due to the fatty acid chains extending luminally. It prevents adherence and penetration of bacteria, i.e. it repels luminal content by acting like a closing seal. In ulcerative colitis, but not in Crohn’s disease, the colonic PC content is reduced by up to 70% even when no inflammation is present. The inherent low mucus PC content reduces the surface tension and, thus, impairs the mucosal barrier. In asymptomatic patients the low mucus PC content may be sufficient to maintain a labile intact barrier function. Yet unknown factors may further reduce the mucus PC concentration below a critical threshold where the mucosal barrier is broken followed by precipitation of an inflammatory episode.

It was shown that PC secretion occurs mainly in the small intestine and less in colon. This may be the reason why the clinical manifestation of UC is exclusively in colon. According to the concept that the lack of PC contributes to the pathogenesis of UC we proposed that the local substitution of PC could enhance or even normalize the mucus PC content and re-establish the mucosal barrier. It is suggested that the application of high PC concentrations in the lumen may facilitate its integration into the mucus. It may first distribute within the upper mucus layer from where it may be integrated in the lower, densely packed mucus layer to reconstitute the lamellar PC monolayer for enhancement of the hydrophobic protective barrier against luminal bacteria. An additional aspect of PC supplementation relates to the anti-inflammatory properties of PC. It may partition into the mucosal cell plasma membrane and mediates a decrease of TNF-α secretion with consequent NFκB deactivation. The mechanism of action of PC is not entirely clear, but there is evidence that via PC the p65 subunit can interact with F-actin and, thus, prevent NFκB activation.

For the purpose of colonic delivery of missing PC a delayed release oral formulation was developed. Encapsulation with Eudragit S100 established a pH-dependent release in the distal ileum. It was indeed shown that the colonic mucus PC content increased to normal values. The concept of local supplementation of missing PC in the colonic mucus was evaluated in a first clinical trial in patients with chronic active ulcerative colitis (clinical activity index (CAI) ≥ 4), but without concomitant steroid treatment. In a double-blind, randomized, placebo-controlled study 60 patients were treated for 3 months with delayed release PC
(rPC) or placebo (4 x 0.5 g daily). Ninety percent of the rPC treated patients reached clinical remission (CAI ≤ 3; 16 patients) or showed a ≥ 50% improvement of their clinical activity (median reduction of the CAI by 7 score points) vs. 10% in the placebo group. This was accompanied by a ≥ 50% improvement of the endoscopic activity index (EAI) in 11 patients (median improvement of the EAI by 3 score points) compared to no change in the placebo group. The same was true for improvement of histology which was only observed in the rPC group. The initial median histology score of 3 (IQR 2–4) decreased to 2 (1–2) (p < 0.0001). In parallel quality of life improved by 50% in 16 of 29 evaluated rPC treated patients vs. 2 of 28 patients in the placebo group.

In a dose finding study with chronic active pancolitis patients, CAI improvement started with 1 g rPC daily reaching a plateau at 3 and 4 g rPC daily. This was paralleled by the EAI improvement. The median time to clinical response was 5 (IQR 2–8) weeks. Mild bloating was registered in 40% of patients with no difference between the study groups. Three of 10 patients in the 4 g dose group reported nausea. Other adverse events were not registered.

Even more convincing proof of its clinical efficacy was obtained when the most difficult to treat patient population of steroid-refractory ulcerative colitis was evaluated. At a daily dose of 2 g rPC for 3 months 50% of the patients could be withdrawn from steroids and at the same time achieved clinical remission (CAI ≤ 3) or a ≥ 50% CAI improvement compared to 10% in the placebo treated group. In total, 80% of the rPC treated patients could discontinue the steroid therapy. In parallel with the clinical improvement, 60% of rPC patients fell below an EAI of 4 and 70% had an improved EAI of ≥ 50% compared with only 3% of placebo recipients.

These results of monocentric trials were now confirmed in a recent multicenter phase IIb trial where the clinical activity, optimal dose and occurrences of adverse events were evaluated over a treatment period of twelve weeks.

**In conclusion:** A new concept for the therapy of ulcerative colitis has been developed based on the observation that a lack of phosphatidylcholine (PC) in colonic mucus is of key pathogenetic relevance. It is the logical consequence that insufficient PC content requires substitution to maintain the mucosal barrier. Only delayed release oral PC is able to deliver PC to the distal intestine. Incorporation of PC into the mucus enables to reestablish a hydrophobic barrier as first line of defense.
Topical therapy

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Topical therapy with mesalazine and/or glucocorticoids is standard treatment in patients with distal ulcerative colitis. Opinion varies about the value of topical therapy in patients with distal colonic Crohn's disease, as stated in the ECCO consensus on Crohn's disease in 2010. Mesalazine has a broad spectrum of anti-inflammatory activities. Since mesalazine is acetylated and therefore inactivated when it is taken up by the intestinal mucosa and then further acetylated during its passage through the liver, mesalazine is a topically active compound. Its clinical efficacy therefore correlates with its local concentration in the gut.

Budesonide is a non-halogenated glucocorticoid which is highly lipophilic and shows good tissue penetration with a high receptor binding activity (about 9 times higher than that of dexamethasone). Approximately 90% of absorbed budesonide undergoes first-pass metabolism in the liver which produces almost inactive metabolites. Budesonide thus combines a high topical glucocorticoid activity with a low systemic side effects rate. Rectal mesalazine is more effective than rectally administered systemically-active glucocorticoids or budesonide. In patients with mild to moderately active distal ulcerative colitis topical mesalazine is therefore the treatment of choice. It might be combined with oral mesalazine. Dose-finding studies showed that doses of 1 g or higher are equally effective. There is, however, an effect of the time of treatment (4 weeks are better than 2 weeks). The German guidelines recommend to treat patients with proctitis with mesalazine suppositories at a dose of $\geq 0.5$ g/day, patients with distal ulcerative colitis with rectal mesalazine (enema or foam) at a dose of $\geq 1$ g/day, eventually combined with oral mesalazine at a dose of $\geq 3$ g/day. Even in patients with extensive ulcerative colitis the combination of oral mesalazine ($\geq 3$ g/day) with rectal mesalazine is recommended, since the combined treatment leads to a faster relief of symptoms. Rectal budesonide shows efficacy in patients with distal ulcerative colitis. The standard dose is 2 mg/day, which usually leads to no glucocorticoid-associated side effects. Rectal budesonide is slightly less effective than rectal mesalazine. This has been shown by meta-analyses as well as by direct comparative studies. Thus far, only one study compared rectal mesalazine with a rectal topical glucocorticoid (beclomethasone) and the combination of both. The combination treatment was more effective than mesalazine or beclomethasone alone.

A potential problem of topical rectal treatment is patients adherence. Indeed, the acceptance of rectal therapy is worse in patients with active disease. To improve adherence, rectal foam formulations of mesalazine and of budesonide have been developed. Randomized controlled clinical trials showed a good efficacy and patient acceptance of low-volume foam formulations of mesalazine and of budesonide.

In conclusion, topical treatment with rectal mesalazine and/or budesonide is effective in patients with distal ulcerative colitis. Foam formulations are usually better tolerated than enemas, especially in patients with active inflammation. For reasons of efficacy and safety topical treatment should therefore always be considered in patients with mild to moderately active distal ulcerative colitis.
The inflammatory bowel diseases, and particularly Crohn’s disease, may be understood as progressive disorders in which bowel damage accumulates over time. Although the rate of bowel damage may vary greatly among individuals, nearly all studies of medical therapy in early IBD indicate higher rates of response and remission than in patients with longer disease duration. Early IBD has been defined as disease within 2 years of onset, and post hoc analyses of biologic therapies, including infliximab, adalimumab, certolizumab pegol and natalizumab, demonstrate higher likelihood of response in early disease. The decision to start immune suppression or biological therapy soon after diagnosis is a difficult one, as patients do not have a long experience with the disease, and the ability to predict the course of disease is imperfect. Studies of early aggressive therapy now include mercaptopurine for children with first exposure to corticosteroids for Crohn’s disease, the step-up/top-down study comparing conventional therapy to early induction with infliximab and maintenance with immunomodulator in Crohn’s disease, and comparisons of combination therapy with infliximab and azathioprine with monotherapy with either drug for Crohn’s disease (SONIC) and ulcerative colitis (UC-SUCCESS). These studies demonstrate robust benefit for early therapy with these effective agents, and also suggest that combination therapy, surprisingly, does not increase the risk for serious infection in Crohn’s disease. Further studies are needed to determine optimal drug regimens tailored to individual risk in inflammatory bowel disease.
Session VII

The classical immunosuppression: Overrated or underused?
Mucosal healing: A new treatment goal or old wine in new skins

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Evidence supporting the importance of mucosal healing (MH) in IBD has increased in the last years. This was the case for decades in ulcerative colitis (UC), but not in Crohn’s disease (CD). The main reason was that early studies from the GETAID found that endoscopic pattern severity correlated poorly with clinical and biological activity, and that clinical remission obtained with steroids was associated with MH in only one third of CD patients. Also, the degree of endoscopic improvement in patients with prednisolone-induced clinical remission did not predict the subsequent clinical course in terms of steroid weaning, or occurrence of relapse. Thus, achievement of MH by steroids was not sufficient to impact on the outcome. The picture is different when considering maintenance therapies such as immunosuppressants or biologics. MH can be achieved and maintained in CD with thiopurines and anti-TNF agents. Recent studies suggests that MH predicts a generally favorable outcome of disease based on all types of treatment strategies, except corticosteroids, and is related to treatment efficacy, reduced frequency of surgery and hospitalizations. MH may also predict the risk of relapse after infliximab discontinuation in patients in remission on combined maintenance therapy. We are still lacking a validated definition of mucosal healing. In clinical practice, we do not know if a complete MH must be obtained to improve the outcome or if a partial healing is sufficient. In UC, MH can be obtained with several types of drugs, including 5-aminosalicylates, steroids, azathioprine, methotrexate, infliximab and adalimumab. Data from several studies suggest that MH may be associated with a better outcome in UC, more specifically a decreased risk of relapse and a reduced risk of surgery. Also, better control of inflammation, as demonstrated with mucosal healing, may be associated with decreased risk of colorectal cancer. MH is becoming the gold standard as therapeutic objective in IBD. Ongoing studies are assessing the potential benefit of therapeutic algorithm based on endoscopic improvement.
Deep remission: A new concept?

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Crohn’s disease (CD) is a chronic inflammatory disorder of the large and small intestines characterized by periods of clinical remission alternating with periods of relapse defined by recurrent clinical symptoms. However, even during periods of clinical remission, laboratory or endoscopic evidence of persistent inflammation can be seen, as evidenced by elevation of serum and fecal inflammatory biomarkers and/or abnormal endoscopic and radiographic imaging. Persistent inflammation is believed to lead to progressive bowel damage over time, which manifests as the development of disease complications such as strictures, fistulae, and abscesses. These disease complications frequently lead to a need for surgical resection, which in turn leads to disability. Thus, CD can be characterized as a chronic, progressive, destructive, leading to disability. In RA, treatment paradigms have evolved beyond partial symptom control alone toward the induction and maintenance of sustained biological remission, also known as a “treat-to-target” strategy, with the goal of improving long-term disease outcomes (reduced structural damage and disability). In CD, there is currently no accepted well-defined, comprehensive treatment goal that requires the treatment of both clinical symptoms and biologic inflammation.

It is important that this treatment concept begins to evolve for CD. A treatment strategy that delays or halts progression of CD towards increasing complications and disability is a priority. As a starting point, a working definition of sustained deep remission that includes long-term biological remission and symptom control, with defined patient outcomes including no disease progression has been proposed. The proposed definition of sustained deep remission for patients with early CD is clinical remission (CDAI < 150 points for patients in clinical trials or absence of symptoms in clinical practice), mucosal healing, CRP < 5 mg/L, and fecal calprotectin < 250 μg/g maintained for ≥ 1 year. In patients with established CD, symptom improvement (150 < CDAI < 220 points in clinical trials and improvement of inflammatory symptoms in clinical practice), mucosal healing, CRP < 5 mg/L, and fecal calprotectin < 250 μg/g over the same timeframe could be targeted. The concept of sustained deep remission represents a goal for CD management that may still evolve. Treatment algorithms that “treat to target” will be needed to achieve sustained deep remission, but remain to be defined. Clinical trials are needed to evaluate whether treatment algorithms that tailor therapy to achieve sustained deep remission in patients with CD can prevent disease progression and disability. In particular, studies are needed to establish whether achievement of sustained deep remission alters the disease course over time and improves patient outcomes, such as disability and need for surgery.

References:

Methotrexate: Underused and ignored?

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Analyses about the use of methotrexate in inflammatory bowel diseases reveal that this drug is used in a negligible number of patients with inflammatory bowel diseases (IBD) compared to anti-TNF agents or thiopurines. Conversely, methotrexate, which was initially developed in 1948 for the treatment of leukemia, has a successful track record in autoimmune and neoplastic diseases and has been used clinically for nearly 60 years as low and high dose therapy to treat lymphomas, Wegeners’ disease, psoriasis and rheumatoid arthritis. In IBD methotrexate’s clinical efficacy has been established for steroid dependent Crohn’s disease (CD) in adults and also in children, refractory or intolerant to thiopurine therapy. In the North American Crohn’s Study Group’s landmark trial, 39.4% of the patients receiving methotrexate (25 mg, intramuscularly (im)/week) were in clinical remission, compared to 19.1% of the patients in the placebo group, at 16 weeks. Also continued methotrexate treatment (15 mg/im/once weekly) to maintain steroid free remission in CD patients has been shown to be highly effective (after 40 weeks 65% remission in the methotrexate group vs. 39% in the placebo group). In ulcerative colitis the efficacy of methotrexate is currently debated, since the only prospective placebo controlled trial did not reveal significant differences in the clinical outcome between oral methotrexate (12.5 mg/week) and placebo. However, if methotrexate is applied in a similar fashion (subcutaneously or im) and dose (25 mg/week) as in the North American Crohn’s study, several case series suggest that methotrexate could be similar effective in ulcerative colitis patients. Two clinical trials are currently underway to finally proof or refute the efficacy of methotrexate in ulcerative colitis. An investigator-initiated trial in France “Comparison of Methotrexate vs Placebo in Steroid-Refractory Ulcerative Colitis (METEOR)” is currently investigating the efficacy of 25 mg methotrexate / week administered subcutaneously compared to placebo in inducing clinical remission (www.ClinicalTrials.gov; NCT00498589). The second clinical trial “Randomized, double blind, prospective trial investigating the efficacy of methotrexate in induction and maintenance of steroid free remission in ulcerative colitis (MERIT-UC)”, which is funded by the National Institutes of Health (NIH) and supported by the Crohn’s and Colitis Foundation of America (CCFA), started recruitment in February 2012 (www.ClinicalTrials.gov; NCT01393405). Hopefully both of these trials will help to further elucidate the efficacy of methotrexate in patients with active ulcerative colitis in need for an effective and affordable steroid-sparing agent.

References:


Azathioprine in the post-SONIC era: To combine or not to combine – That is the question

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Over the last decade, the use of anti-TNF agents has changed the management of patients with IBD. Infliximab, adalimumab and certolizumab have been successful in inducing and maintaining remission in Crohn’s disease, and later the two first agents have also shown efficacy in ulcerative colitis. As these new agents entered the armamentarium of drugs against IBD, their integration with older therapies, and in particular with immunosuppressors became a focus of attention, as much in terms of added benefits as in terms of added toxicity. Retrospective clinical series as well as post hoc analyses of the early trials have suggested that combination therapy of infliximab and azathioprine could have added benefit to induce steroid-free remission, at least in Crohn’s disease, leading to the prospective use of this combination in patients with early disease, naïve to both therapy, in the pivotal SONIC trial. Very recently a similar trial in ulcerative colitis patients came to the same conclusions. In both diseases, the combination was proven superior to induce steroid-free remission and to maintain this remission, as compared to either drug alone. The safety of the combination therapy has not been a concern in the randomized trials. However, combination therapy with two immunosuppressive agents may potentially be associated with increased risks of infection and malignancy, especially in particular patient populations such as adolescents and elderly. Furthermore, the risk-benefit ratio may not favor combination therapy in patients that have previously failed azathioprine before. Thus, despite the recent evidence, combination therapy may not be the ready-made solution for all patients.
Tacrolimus and cyclosporine

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Tacrolimus (FK506) is an effective immunosuppressive treatment in steroid refractory ulcerative colitis supported by two controlled trials and several larger uncontrolled patient series. Its role in Crohn’s disease is very limited. A substantial percentage of patients fail to respond to the drug and its use is limited by unpredictable toxicity. Tacrolimus is substrate to the metabolizing enzymes cytochrome P450 (CYP) 3A4, CYP3A5 and the multidrug efflux pump P-glycoprotein, encoded by ABCB1 (MDR1). An individual's response to tacrolimus may in part be genetically determined by functional variation of these genes. We have performed a pharmacogenetic evaluation of tacrolimus in UC in 89 patients (79 UC, 10 indeterminate colitis-IC) treated with tacrolimus for steroid refractory disease. All subjects were genotyped for the following polymorphisms: CYP3A4*1B, CYP3A5*3, ABCB1 C1236T, C3435T and G2677T/A. Genotype-phenotype associations were evaluated by univariate and multivariate analyses with respect to efficacy and toxicity of tacrolimus therapy. Forty-seven (57%) patients achieved short-term remission and 11 (12%) achieved short-term response. Twenty-seven (30%) patients failed to respond to therapy. Overall 35 patients (40%) patients experienced side effects. Five patients had to stop therapy due to toxicity (2 = reversible renal impairment, 1 = nausea, 1 = worsening of diabetes mellitus). The most frequent adverse events were tremor (n = 25), hyperglycemia (11), slight and reversible renal impairment (6), paraesthesia (4) and nausea (3). None of the selected candidate SNPs were associated with efficacy or toxicity of tacrolimus therapy.

We also evaluated the efficacy of infliximab-salvage therapy in patients with refractory ulcerative colitis failing to respond to tacrolimus. Twenty-four patients were enrolled in this evaluation. Reasons for tacrolimus therapy were steroid-refractory disease in 19 patients and steroid dependency in 5 patients. All patients receiving infliximab had tacrolimus refractory active disease (Lichtiger score > 10) and were treated with 5 mg/kg at weeks 0, 2 and 6 and every 8 weeks thereafter if tolerated. Six of 24 patients (25%) achieved remission following infliximab infusion and 4/24 (17%) had an initial response only but underwent proctocolectomy later due to loss of response (3) or development of a delayed hypersensitivity reaction (1). Fourteen patients (58%) completely failed to respond with 10 undergoing colectomy. Eight patients experienced side effects under infliximab including two infectious complications (herpes zoster and herpes pneumonia).

We conclude that Infliximab offers a therapeutic option as rescue therapy in about a quarter of patients with active UC. It is unclear whether tacrolimus is superior to infliximab or cyclosporine in active UC.
Session VIII

Biologica ls and beyond
Biological treatment of Crohn’s disease

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Introduction of biological agents for the treatment of Crohn’s disease (CD) has led to a transformation of the treatment paradigm. Several biological compounds are approved for patients with CD refractory to conventional treatment: infliximab, adalimumab, and certolizumab pegol (and natalizumab in several countries including the United States). However, despite the use of biologics for more than a decade questions still remain about the true efficacy and the best treatment regimens – and especially when to discontinue treatment? Furthermore, a need for optimizing treatment with biologics still exists, as up to 40% of patients with CD do not have any relevant response to the existing biological agents (i.e. primary failures). A better patient selection might maximize the clinical benefit while minimizing complications associated with this type of therapy. However, the clinical tools able to identify such patients are still unavailable, but it is evident that for instance CD patients who are smokers might be expected both to have a lower response rate and a shorter duration of response. Further, the trough level strategy may help the clinician to optimize therapy and to avoid loss of response and/or immunogenicity. This course of therapy implies a correct dosage with the lowest antibody level just before the periodical administration of the biological agent (i.e. the trough level) being high enough to exert the full effect of the drug. On the other hand, peak levels and average levels should, however, not exceed concentrations associated with increased toxicity. Randomized, controlled studies focusing on trough levels and antibodies towards the biological agent in routine clinical situations may add important bricks to the puzzle for a more rational treatment algorithm of CD patients. In some situations the risks (i.e. immunogenicity, serious infections and promotion of neoplasia) may, however, not outweigh the benefits of biological treatment.
The first biological therapy that was successful in ulcerative colitis was infliximab, as shown by the ACT I and ACT II studies. Infliximab was effective in moderate to severe ulcerative colitis patients, reduced hospitalization and surgery and induced mucosal healing. Mucosal healing was associated with reduced colectomy rates. The SUCCESS study confirmed that a combination of infliximab and azathioprine was the most effective therapeutic strategy. Infliximab is also effective in reducing colectomy rates in severe hospitalized ulcerative colitis patients. The CYSIF study has confirmed that ciclosporin and infliximab are equally effective in acute severe ulcerative colitis. Recently adalimumab has also been shown in the ULTRA 1 and ULTRA 2 studies to be effective therapy in ulcerative colitis. Further anti-TNF therapies in ulcerative colitis are expected such as golimumab. Emerging therapies include anti-adhesion molecule therapies and signaling molecule inhibitors.

Ulcerative colitis patients who have extensive disease, have been hospitalized or require steroids are at high risk of colectomy and anti-TNF therapy should be considered in such patients. In addition, patients who fail steroids and/or immuno-suppressive therapy also are candidates for anti-TNF therapy. This may avoid colectomy, which is important as patients after colectomy and ileo-anal pouch may continue to have frequent diurnal and nocturnal bowel movements, incontinence, and social restrictions. In addition, loss of fecundity in women of reproductive age group, pouchitis and pouch failure remain potential long term problems after pouch surgery.
Stem cell transplantation: The ASTIC Trial

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Background: The Autologous Stem Cell Transplantation International Crohn's Disease (ASTIC) Trial is a randomised controlled evaluation of the proposition that immunoablation and hemopoietic stem cell transplantation improves the course of Crohn’s disease. Recruitment of all 48 patients in the trial will complete in early 2012 and results to date are presented descriptively here.

Methods: Patients with impaired quality of life due to active Crohn’s disease, despite at least 3 immunosuppressive agents all receive mobilisation treatment (iv cyclophosphamide 4 gm/M2 over two days followed by recombinant human granulocyte-colony stimulating factor [GCSF, filgrastim], 10 µ/kg daily before randomisation to immediate (1 month) or delayed (1 year) immunoablation and stem cell transplantation. The conditioning regime is iv cyclophosphamide 50 mg/kg per day for 4 days, anti-thymocyte globulin 2.5 mg/kg/day and methyl prednisolone 1 mg/kg on days 3–5. The bone marrow is reconstituted by infusion of an unselected graft of 3–8 x 10^6/kg CD34 positive stem cells. Results are compared one year after mobilisation alone or after transplantation.

Results: Twelve months after stem cell transplantation (early or delayed) the Crohn’s Disease Activity Index (CDAI) fell from 324 (median, interquartile range 229–411) to 161 (85–257, n = 17) compared to 351 (287–443) to 272 (214–331) following mobilisation alone (n = 11). Six patients had a normal CDAI after transplantation vs one after mobilisation. C reactive protein fell from 16.6 (6.7–32.0) mg/l to 6.5 (3.5–12.5) mg/l vs 14 (8.0–27.0) mg/l to 9.0 (2.0–23.4) mg/l following mobilisation alone. The Crohn’s Disease Endoscopic Index of Severity (CDEIS) (aggregate for upper and lower endoscopy) fell from 18 (10–25) to 5 (1–11) following transplantation vs 14 (12–16) to 9 (4–22) following mobilisation. Three patients achieved the goal of a normal CDAI, no drug therapy and normal upper and lower endoscopy one year after transplantation but so did one patient following mobilisation alone. Serious adverse events were common (n = 100 to date) with 42 infective episodes requiring or prolonging hospitalisation, following both mobilisation and conditioning and transplantation. There were 7 episodes of viral (re)activation. Temporary flare of Crohn’s disease activity or a need for surgery occurred in 8 patients.

Conclusions: Immunoablation and hemopoietic stem cell transplantation appears to be an effective treatment for some patients with Crohn’s disease, although full results will be required for a firm conclusion. Risks are significant, making it potentially suitable for only a limited number of patients. Data from the whole trial will be needed to judge whether mobilisation alone has any benefits.
Strategies against adhesion molecules

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An emerging approach to the treatment of inflammatory diseases is inhibition of leukocytes migration into inflamed tissue by blocking cellular adhesion molecules. Integrins are heterodimeric proteins that regulate cellular movement. The α4β7 integrin that is primarily involved in the recruitment leukocytes to the gut is present on the surface of a small population of circulating T-lymphocytes. The major ligand for α4β7, MAdCAM is selectively expressed on the endothelium of the intestinal vasculature and is present in increased concentration inflamed tissue. In addition lymphocytes also express alpha 4 beta 1 receptors that interact with vascular cellular adhesion molecule 1 (VCAM-1). This latter molecule is widely expressed on vascular endothelium in most tissues. Therefore, interference with this interaction theoretically could result in relatively non specific interference with lymphocyte trafficking.

Several drugs that exploit these mechanisms have shown efficacy in randomized controlled trials. In Crohn's disease, natalizumab, a CDR-grafted humanized antibody directed towards the Alpha monomer has been shown to be effective for both induction and maintenance of remission.

Two recent controlled trials evaluated natalizumab as induction and maintenance therapy in patients with active Crohn’s disease. In ENACT I, 905 patients were randomly assigned to receive 300 mg of natalizumab or placebo at weeks 0, 4, and 8. The primary outcome was response, defined by a decrease in the Crohn’s Disease Activity Index (CDAI) score of at least 70 points, at week 10. In ENACT II, 339 patients who had responded to natalizumab in the first trial received 300 mg of natalizumab or placebo every four weeks through week 56.

In ENACT I, the natalizumab and placebo groups had similar rates of response (56% and 49%, respectively; P = 0.05) and remission (37% and 30%, respectively; P = 0.12) at 10 weeks. In ENACT II continued treatment with natalizumab resulted in response rates of (61% vs. 28%, P < 0.001) and remission rates (44% vs. 26%, P = 0.003) through week 36 than did switching to placebo.

In ulcerative colitis, a Phase II clinical trial evaluated two doses of a humanized antibody, MLN-02 directed to the α4β7 integrin against placebo. 181 patients with active ulcerative colitis who had failed 5-ASA or no treatment were evaluated. Six weeks after the administration of two infusions of drug or placebo, approximately one-third of patients who received the antibody were in remission as compared to 14% of those who receive placebo (P = 0.03). Parallel improvements in endoscopy, histopathology and health-related quality of life were also demonstrated. Therapy was well-tolerated and there was no evidence of opportunistic infection.

Two randomized controlled trials have also evaluated the use of anti-sense to VCAM-1 in patients with ulcerative colitis with favourable results.
Recently the risk of progressive multifocal leukoencephalopathy (PML) has been an important limitation to the use of natalizumab. This uncommon, progressive neurological disorder is frequently seen in patients who are severely immunocompromised (HIV, malignancy). The disease is related to reactivation of a Papova virus (JCV) that is latent in approximately 60% of the population. This disturbing occurrence has resulted in very limited use of natalizumab for the treatment of Crohn’s disease.

Notwithstanding the seriousness of PML, it is highly likely that current toxicity concerns can be dealt with through additional research and that more selective adhesion molecule inhibitors will ultimately become valuable agents for the treatment of IBD. In support of this notion data from a recent Phase III trial of vedolizumab in ulcerative colitis have confirmed this to be an effective molecule that selectively reduces gut inflammation. Multiple other agents that exploit this concept are currently under development.

References:


State-of-the-Art Lecture IV

The future of IBD therapy: Where do we go from here?

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There are six important trends that will impact the future of IBD therapy. 1) Increased use of biomarkers and imaging. An important minority of patients with an established diagnosis of Crohn’s disease and symptoms compatible with a disease flare do not have evidence of active Crohn’s disease by laboratory, endoscopic, and radiographic criteria. Studies demonstrate a weak to absent correlation between clinical symptoms as measured by the Crohn’s disease activity index (CDAI) and endoscopic findings as measure by the Crohn’s disease endoscopic index of severity (CDEIS) as well as other biomarkers such as CRP and fecal calprotectin and lactoferrin. Up to 18% of patients with Crohn’s disease and moderate to severe clinical symptoms have no evidence of ulceration at colonoscopy. 2) Increased use of pharmacokinetics to customize drug dosing for individual patients. Multiple factors impact the pharmacokinetics of monoclonal antibodies including the presence of anti-drug antibodies, concomitant immunosuppression, high baseline antigen production and concentrations (increased tumor necrosis factor production for example), low serum albumin, high inflammatory burden (increased CRP concentrations for example), large body mass index, and male gender. Customized dosing regimens that adjust for these clinical factors in order to achieve blood concentrations of drug that are associated with optimal clinical outcomes will be developed and come into common clinical practice. 3) Evolution of treatment endpoints from symptoms to mucosal healing. Mucosal healing will become part of the treatment goals for ulcerative colitis in order to optimize clinical outcomes and to minimize colectomy rates. In Crohn’s disease, treatment goals will evolve to prevention of bowel damage and surgery. Patients will be treated to deep remission (both clinical remission and mucosal healing), with the goals of preventing bowel damage and surgery in the short to intermediate term, and preventing disability in the longer term. 4) Evolving data from several large randomized trials will demonstrate that azathioprine monotherapy is minimally effective as a disease modification agent in Crohn’s disease, and use of azathioprine as monotherapy will decline. 5) Increased use of combination therapy. Prospective randomized controlled trials have demonstrated that combination therapy with azathioprine and infliximab is superior to monotherapy with either agent. Combination therapy leads to optimized clinical outcomes and will be an important part of strategies to achieve treatment goals like mucosal healing and prevention of bowel damage and surgery. 6) Discovery of novel therapeutic agents. There is a rich pipeline of novel therapeutic agents. Treatment strategies that appear particularly appealing include selective anti-integrin therapy with anti alpha 4 beta 7, anti beta 7, and anti MadCam-1 antibodies, anti-interleukin 12/23 therapy with ustekinumab, and Janus kinase 3 (JAK 3) inhibition with tofacitinib.
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Non-invasive evaluation of ulcerative colitis in remission – Correlation with endoscopy

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Introduction: Ulcerative colitis (UC) patients being in clinical remission may have inflammatory endoscopic and histological activity, but the discomfort and costs of regulate control colonoscopy requires finding other surveillance parameters.

Methods: 28 patients with UC in clinical remission, with both lower and pancolic forms, were tested for serum C reactive protein (CRP – qualitative screening) and fecal calprotectin (qualitative immunochromatographic test). They performed diagnostic colonoscopy, noting the Mayo activity score.

Results: CRP was positive in 7 patients (cut-off 0.6 mg%), representing 25% of all, while calprotectin was positive in 15 patients (cut-off 60 µg/g) – 53.57%. In colonoscopic examination normal aspect (Mayo 0) was seen in 14 patients (50%), Mayo 1 in 10 patients (35.71%) and Mayo 2 in 4 patients (14.29%). Considering endoscopic activity as reference, fecal calprotectin was positive in 13 of 14 patients with 1 or 2 Mayo activity (sensibility 92.86%, specificity 85.7%), while CRP had a sensibility of 42.85% and specificity 92.86%. Correlation coefficient of fecal calprotectin value with endoscopic activity was 78.77%, and CRP’s of only 41.24%.

Discussion/Conclusion: Many UC patients in clinical remission present signs of activity in colonoscopic examination. Those patients relapse more frequent and develop more complications, requiring a much aggressive therapeutic approach. Of the non-invasive markers tested, fecal calprotectin has a better correlation with Mayo endoscopic score than serum CRP and might be useful as a surveillance parameter.

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Effect of dietary extracts on the Toll-like receptor pathway: Working towards personalized nutrition for inflammatory bowel disease

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Background: Nutrigenomics New Zealand (NZ) is a scientific initiative that seeks to interpret nutritional requirements in relation to genetics. The primary focus of this initiative is Inflammatory Bowel Disease (IBD). It is known that there is a strong link to a range of genetic susceptibilities in IBD and as a consequence dietary requirement for IBD sufferers varies considerably. One of the leading theories on the etiology of this disease is an overactive immune response to bacteria and other foreign antigens, resulting in increased inflammation. An important signaling cascade implicated in IBD, and involved in intestinal detection of foreign antigens, is the TLR (Toll-like receptor) pathway. To investigate the effect of diet on the TLR pathway, we have developed an assay to screen fruit extracts for anti-inflammatory properties specifically through TLR signaling.

Method: An assay was developed using HEK-Blue hTLR4 and HEK-Blue hTLR2 cells to screen compounds for anti-inflammatory properties using NF-κB expression as a marker of inflammation. A range of fruit extracts that have been identified in the literature with high plant polyphenol content are being screened to see their effect on TLR4 and TLR2 ligand-stimulated NF-κB expression.

Results: Some of the screened extracts reduced the effects of ligand-induced NF-κB expression through the TLR4/2 pathway.

Conclusion: Preliminary results show that some fruit extracts exhibit anti-inflammatory properties through the TLR4/2 pathway. Further studies using these extracts can give us better insights into the mechanism of action between diet and disease interactions for a subset of the IBD population. If these results can be replicated in human and animal models of IBD then this has the potential of contributing to the development of personalized nutrition for IBD sufferers which will help in better management of disease.
Retrospective analysis of microscopic colitis patients: Single center experience

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Introduction: Microscopic colitis (MC), a cause of chronic diarrhea has been increasingly recognized during the past years, but still is an underestimated diagnosis. As colonoscopy appears normal diagnosis depends on the awareness of the physicians.

Methods: Between January 2010 and December 2011, biopsy-proven microscopic colitis patients with normal colonoscopic findings were evaluated for demographic and laboratory data, treatment and outcome. Blood biochemistry, hematological counts, thyroid-stimulating hormone, antigliadin IgA-IgG, antiendomysium IgG, parasitic and bacterial stool tests and stool culture were obtained at diagnosis from all patients.

Results: There were 34 patients (18 male, 16 female) with mean age 54.3 years. The mean subepithelial collagenous band thickness was 23.7 µm [range 10–62.6 µm]. There was no marked abnormality in laboratory data, celiac tests were negative, stool tests were normal. Four patients had thyroid, 2 patients had rheumatologic disease. In 5 patients data was missing or were lost to follow-up. 17 patients received Budesonide (9 mg/day for 8–12 weeks), 7 patients received antidiarrheal or spasmolytic agents, 1 patient was already on steroid treatment, 4 patients received no treatment because by the time of diagnosis symptoms had been regressed spontaneously. In all patients symptoms resolved/improved within the first two weeks after initiating therapy, but relapsed in 2 patients after discontinuing. No serious side effects were seen with treatment. Nine patients agreed to undergo a colonoscopy with biopsy after treatment; histological remission was confirmed in all except one.

Discussion/Conclusion: MC is a benign but chronic disease that interferes with quality of life and has a low spontaneous resolution rate. It should be remembered as a cause of chronic diarrhea. Among others budesonide is one well known treatment option, with high success rate and low side effects. As relapse can be seen, long term follow up may be needed.
Postoperative fistulas on the neo-terminal ileum in Crohn’s disease

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Introduction: Postoperative recurrence in Crohn’s disease (CD) is expected in approximately 40% of patients at the first year and 80% at five years. The appearance of a fistula on neo-terminal ileum is a rare event; its incidence is not well appreciated. The objective of this study was to determine the features of patients with ileal or ileocolonic CD who had undergone surgical bowel resection and presented a postoperative fistula in the neo terminal ileum.

Methods: A retrospective study compiled records of patients treated in the Gastroenterology A Department between 1990 and 2010, with ileal or ileocolic CD who underwent ileal resection.

Results: Twenty-two patients were included (14 males and 8 females). The mean age was 40 years (range 25–64). Sixteen patients (73%) had ileo-colonic disease and six patients (27%) had ileal disease. Perianal disease was present in 4 patients (18%). Extraintestinal manifestations were reported in 50% of cases (rheumatic manifestations in 8 cases, primary sclerosing cholangitis in 1 case and pyoderma gangrenosum in 1 case). The median delay between CD diagnosis and surgery was 4.6 years. Indications for surgery were symptomatic ileal stenosis (59%), penetrating disease (13%), failure of medical treatment in severe acute colitis (5%) and pseudo-tumor disease (5%). Post operative preventive treatment was initiated in 11 patients (50%). Eight were on azathioprine and 3 on 5-aminosalicylates. The delay of appearance of fistulas after surgery was 8 years. There were ileo-ileal, ileocolic and enterocutaneous fistulas respectively in 9, 4 and 9 cases. Treatment was surgical in 77% of cases, 13% of patients were treated with antibiotics and 9% with anti-TNF alpha. Mean follow-up was 30 months. Recurrence of the fistula was noted in one patient.

Discussion/Conclusion: In our study, 50% of patients were on prophylactic treatment after small bowel resection. Most of them had stricturing phenotype. Postoperative fistulas were in the majority of cases associated with stenosis.
Azathioprine withdrawal in patients with Crohn’s disease in clinical remission: Results of a randomized prospective trial

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Introduction: Durable remissions have been achieved in many IBD patients on azathioprine or 6-mercaptopurine, but the duration of treatment and identifying which patients may stop therapy is yet unresolved. The question of whether treatment with azathioprine can be safely interrupted after a period of prolonged remission is of great interest to patients and physicians because most patients affected by Crohn’s disease are young, have a long life expectancy, and often have concerns about the long-term safety of the drug.

Aim: To prospectively evaluate the relapse risk after AZA withdrawal at 18 months in patients with Crohn’s disease and who were in remission during at least 36 months.

Methods: It is a prospective randomized clinical trial conducted in Gastroenterology A Department of La Rabta Hospital. Patients were included between January 2009 and July 2009. Inclusion criteria were a Crohn’s disease in clinical remission for at least 36 months on azathioprine or 6-mercaptopurine with any flare during this period and an age over eighteen. Immunosuppressive therapy was indicated for a luminal disease. Exclusion criteria were: extended ileal disease, multiple intestinal resections. No other treatment was permitted. Patients were randomized according to gender, age, location of disease (Montreal classification), indication of immunosuppressive therapy, duration of treatment and concomitant perineal disease to either continue their treatment or to stop it. Clinical and biological monitoring performed at baseline and then every 3 months, included calculation of the CDAI score and biological tests (blood counts, sedimentation rate, C-reactive protein). We prospected to include 60 patients, but inclusions were stopped after enrolling 32 patients because of negative results.

Results: Fourteen patients were randomly assigned to continue azathioprine (group 1) and 18 to stop it (group 2). Characteristics of patients at entry were similar in the 2 study groups. At 18 months, 1 patient from group 1 had relapsed and 7 from group 2 (p = 0.02, HR = 7.3, IC 95% 1.2–20.3). Among group 2 patients, no clinical or biological predictive factor of relapse was identified. One patient from group 2 with ileal Crohn’s disease presented 10 months after stopping azathioprine an ileo-cutaneous fistula and was treated surgically. The 6 remainder relapsing patients were retreated by azathioprine alone in 2 patients and associated with aminosalicylates in 2 patients and corticosteroids in 2 patients. Five patients up to successful remission, but one had a rescue subtotal colectomy because an acute severe flare resistant to intensive medical treatment. The study was stopped because of the high risk of relapse in group 2 and the need of surgical resections in 2 patients.

Discussion/Conclusion: Our results confirm that AZA withdrawal is associated with a high risk of relapse and surgical intervention, whatever the duration of remission under this treatment. The new here is that there isn’t any predictive factor of relapse in contrast with the study of the GETAID and that retreatment with azathioprine is not always efficient.
Exclusive perineal Crohn’s disease: Epidemiological and evolutive features

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Introduction: Perineal Crohn’s disease represents an independent clinical entity. In this form, lesions are particularly complex, relapsing and difficult to treat. The aim of this study was to assess clinical, radiological, therapeutic and evolutive characteristics of this form.

Methods: We conducted a retrospective study including 689 consecutive patients with Crohn’s disease managed in the gastroenterology A department of La Rabta hospital between 2000 and 2010. Patients with perineal Crohn’s disease without luminal involvement were colligated. Clinical, radiological, therapeutic and evolutive features of these patients were reviewed.

Results: Among the 689 patients, 9 (1.3%) had exclusive perineal location. They were 2 women and 7 men, with mean age of 46.5 years. Revealing symptoms were perineal swelling in 88.8%, perineal abscesses in 44%, perineal ulcers in 22% and asymptomatic stricture in 22%. MRI imaging was carried out at all patients. It showed transphincteric tract of fistulae (stage 2 of Parks), suprasphincteric tract (stage 3 of Parks), and extrasphincteric tract (stage 4 of Parks) in respectively 4, 1 and 4 patients. Fistula involving genital tract were noted among 3 patients (2 scrotal and 1 vaginal). Biopsy of ulcers or fistulous tract was realized for 6 patients. Histological examination highlighted one granuloma in half of cases. All patients have received medical treatment. Antibiotic therapy associating ciprofloxacin and metronidazole were conducted in all cases. Immunosuppressive therapy was prescribed for 6 patients (thiopurines n = 2, infliximab n = 4). Surgical drainage with setons was realized for all patients. Remission attested by disappearance of inflammatory signs at MRI was achieved in 5 cases. Mean follow up was 14.33 months.

Discussion/Conclusion: Exclusive perineal Crohn’s disease is rare in our cohort. Diagnosis was largely improved by MRI imaging. Therapeutic strategy is not consensual but it seems to be largely improved by anti TNFα agents.
Outcomes of Crohn’s disease asymptomatic strictures on infliximab therapy: Results of a monocentric experience

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Introduction: Infliximab is generally avoided when patients showed intestinal strictures in Crohn’s disease (CD) because of the risk of occlusive symptoms. However this treatment is more prescribed nowadays in this condition. The aim of this study was to assess becoming of intestinal strictures symptomatic or not under infliximab therapy.

Methods: We conducted a retrospective study in our Department Gastroenterology A of La Rabta Hospital between 2000 and 2010 including consecutive patients with CD for whom infliximab treatment was conducted. We studied cases showing intestinal asymptomatic strictures attested by endoscopy and/or radiology before infliximab therapy.

Results: 82 Patients with Crohn’s disease were treated by infliximab. Twelve (8.5%) had asymptomatic intestinal strictures. They are 5 woman and 2 men. Mean age was 39.5 years [22–50]. CD location was ileal in 5 cases, a ileocolonic in 7 cases. Treatment indication was refractory luminal CD in 25% and complex perineal fistula in 75% of patients. Concomitant prescribed drugs were: corticosteroids (n = 1), thiopurines (n = 8), aminosalicylates (n = 2) and antibiotics (n = 8). Intestinal strictures were spontaneous among 5 patients and anastomotic among the other 7 patients. At Computed tomography, stricture was single in 33% of patients. It showed inflammatory signs in 58.3%. It was more than 5 cm in 8%. During infliximab therapy, stricture became symptomatic in 5 patients (abdominal pain n = 2, occlusive symptoms n = 3). Three symptomatic strictures had been treated (corticoids n = 1, endoscopic balloon dilation n = 1 and surgical resection n = 1). Infliximab was continued in all patients. No one of the 3 treated patients relapsed occlusive symptoms with a mean follow up of 38 months [36–72].

Discussion/Conclusion: Infliximab therapy can worsen preexistent intestinal strictures. Systematic detection of intestinal stenosis before treatment would be useful in order to recognize clinical monitoring.
Serum nitric oxide levels as a marker of inflammation and disease severity in patients with ulcerative colitis: A prospective single center study

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Introduction: Inflammatory bowel disease, or IBD, is a collection of systemic diseases involving inflammation of the gastrointestinal (GI) tract of unknown etiology. The clinical course is characterized by remission and relapse which may develop spontaneously or in response to medical treatment. Since effective medical therapy diminishes mortality in patients with severe UC, determination of inflammatory activity is therefore crucial for the assessment of disease activity and also for the tailoring of therapy. Nitric oxide (NO) as a mediator of cytokine activation, is produced by cytokine-activated cells in inflammation. It is a highly reactive molecule that possesses pro- and anti-inflammatory effects depending on its concentration and the release source. Although conflicting results exist in literature depicting the role of NO in UC, the present study is designed to evaluate NO synthesis and metabolism in patients with UC and to ascertain its usefulness for differentiating active from inactive states of UC.

Methods: The present study was carried out in Ankara Education and Research Hospital, Department of Gastroenterology between March 2010 and May 2011. Forty-two patients with UC (22 in active state, 19 in remission) and 18 healthy controls were included to this prospective study. The disease activity in UC patients was assessed by true love and witts criteria. NO metabolites (NOx), and conventional inflammation markers were determined. NO was estimated as nitric oxide metabolites nitrite/nitrate (NOx) by Griess reaction after conversion of nitrate to nitrite by nitrate reductase, using the commercially available Nitric Oxide Assay Kit from Cayman Chemical Company. The measurement of the total endogenous nitrite concentration was taken as an indirect measurement of NO produced by the peripheral blood monocytes. Correlation analysis was also performed between NOx and other inflammation markers of UC (white blood cell, hsCRP, sedimentation rate).

Results: In patients with active UC serum NOx were found to be significantly elevated compared with inactive UC and healthy controls (p = 0.001 and p < 0.001 respectively). The mean NOx levels in patients with active UC, inactive UC and healthy controls were found to be 3.21 ± 2.73, 0.87 ± 1.15 and 0.79 ± 0.90 µmol/l respectively (Figure 1). The receiver operating characteristic analysis showed that a NOx level of 1.1 was the best cut-off value for predicting active disease with a sensitivity of 81.8% and a specificity of 84.2% (area under the curve (AUC) = 0.806, NPV: 80%, PPV: 85.7%) Correlation analysis suggested that only hsCRP levels were correlated with NOx levels for differentiating active from inactive disease (rs = 0.717 p < 0.001) (Figure 2).
Discussion/Conclusion: Serum NO associated tissue injury might be associated with continuous inflammation of the colonic mucosa by creating a local environment that is enriched with reactive oxygen species, cytokines and other growth factors which may promote endothelial cell apoptosis. Intestinal mucosal damage mediated by NO-associated processes should be considered in the etiopathogenesis of ongoing inflammation especially in severe cases. Moreover serum NO levels may be used as an adjunctive marker to identify active from inactive disease.

Figure 1: Box-plot representation of nitric oxide levels in patients with ulcerative colitis (active and inactive) and healthy controls.

![Box-plot representation of nitric oxide levels in patients with ulcerative colitis (active and inactive) and healthy controls.](image)

Figure 2: The correlation between nitric oxide metabolites (NOx) and hsCRP in patients with active ulcerative colitis. Lines representing the 95% confidence interval (CI) and the 95% prediction interval of the regression line are demonstrated.

![The correlation between nitric oxide metabolites (NOx) and hsCRP in patients with active ulcerative colitis. Lines representing the 95% confidence interval (CI) and the 95% prediction interval of the regression line are demonstrated.](image)
Metabonomics of human fecal extracts characterize the gut microbiota in inflammatory bowel disease

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Introduction: A dysregulation of the colonic microbiota and their metabolites are involved in the pathophysiology of IBD, but the understanding of this interplay remains inadequate. The aim of this study is to employ 1H NMR spectroscopy-based metabolic profiling of fecal extracts in order to characterize and possibly differentiate the complex mixture of metabolites from controls, CD, and UC patients.

Methods: Fecal water extracts from 120 subjects: 33 active CD (HB score ≥ 5), 20 inactive CD, 26 active UC (Mayo score ≥ 2), 20 inactive UC, and 21 controls. A 1H NMR spectrum was obtained from each sample (DRX-600 spectrometer, Bruker Biospin, Rheinstetten, Germany). Data analysis was performed by orthogonal-projection to latent structure-discriminant analysis (SIMCA-P+12, Umetrics, Umeå, Sweden).

Results: A range of amino acids (isoleucine, leucine, valine, lysine, alanine, tyrosine, phenylalanine, and glycine) were significantly increased in active IBD vs. controls, as was succinate and lactate in active CD and UC, respectively. The short chain fatty acids (SCFA) butyrate and propionate were significantly lowered in active IBD vs. controls. Differentiation between CD, UC and controls was possible due to their unique metabolic profiles.

Discussion/Conclusion: The increase of amino acids in active IBD might be the consequence of malabsorption, whereas the disruption of microbiota-related metabolites (SCFA, succinate, and lactate) suggests a dysbiosis of the normal bacterial ecology. The distinct metabolic profiles of CD and UC indicate diverse types of dysbiosis, possibly reflecting their different pathogenesis.
Pulmonary actinomycosis and tuberculosis in a CD patient treated with adalimumab: A case report

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Introduction: Tumor necrosis factor-α (TNF) blockers are highly effective treatment for Crohn’s disease (CD) and are becoming increasingly more used. However, opportunistic infections still remain a major concern due to the profound immunosuppression. We report a case of a CD patient treated with adalimumab who subsequently developed tuberculosis and pulmonary actinomycosis.

Case report: A 23-year-old male was diagnosed with ileal CD at the age of 17 and perianal disease developed several years later. From 2007 patient was maintained on azathioprine with good control of disease. In November of 2010, at the age of 23, he was admitted to our unit with a severe flare. After exclusion of tuberculosis on the basis of normal chest X-ray, negative PPD test and a negative Quantiferon assay therapy with adalimumab was instituted with good clinical response. In January of 2011, one week after third dose of adalimumab patient was hospitalized for influenza A and adalimumab therapy was stopped. In March, the patient was hospitalized for increased number of stools and a dry cough. Chest X-ray and CT scan of the thorax revealed an inflammatory infiltrate in the apical segment of the left lower lobe of the lung without signs of tissue destruction. Bronchoscopy was performed and BAL was positive for Actinomyces meyeri. Staining of the lavate was negative for mycobacteria, however cultures were positive for Mycobacterium tuberculosis both in the lavate and in the stool. The patient was treated with penicillin for actinomycosis and in parallel antituberculous therapy with was instituted (combination of isoniazid, rifampicin and etambutol). Patient had an excellent response to treatment and is currently doing well.

Discussion/Conclusion: This is the first report of pulmonary actinomycosis as a complication of anti-TNF therapy in CD. Our case emphasizes the need for maintaining a high level of suspicion for opportunistic infections in patients receiving anti-TNF treatment.
Tissue-specific overexpression of the transcription factor Nrf2 increases mucosal inflammation upon dextran sulphate sodium treatment

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Background: The transcription factor NF-E2-related factor 2 (Nrf2) is a major modulator of the cellular antioxidative response. So far its cytoprotective function has been well established, yet some recent studies indicate negative effects of Nrf2 overexpression.
In inflammatory bowel disease the regulation of reactive oxygen species (ROS) production and detoxification is of high interest, because the mucosa of patients is infiltrated by macrophages leading to a massive production of ROS. The oxidative burst contributes to tissue destruction and epithelial permeability, but at the same time it is also an essential part of the antibacterial defense.

Methods: To study the influence of tissue specific overexpression of Nrf2 on mucosal inflammation we used transgenic mice conditionally expressing a constitutively active form of Nrf2 (caNrf2) driven by a CMV enhancer. By crossing CMVcaNrf2 mice with either VilCre or LysM Cre mice, activation of caNrf2 expression was achieved in epithelial cells (VilCre-CMVcaNrf2 mice) or in the myeloid cell lineage (LysMCre-CMVcaNrf2 mice) respectively. As control animals mice were used carrying the VilCre- or LysMCre-allele, only.
Acute colitis was induced by administration of 2.5% dextran sulphate sodium (DSS) in the drinking water in Nrf2-overexpressing and control animals for seven days. On day eight, colonoscopy was performed and animals were euthanized. Severity of inflammation was determined by weight loss, shortening of the colon and scoring of hematoxilin and eosin stained section of the colon. As a further parameter we determined myeloperoxidase (MPO) activity in colon samples.

Results: Mice overexpressing Nrf2 in epithelial cells lost 11.6 ± 0.8% of their body weight during colitis, whereas control animals showed a significant lower weight loss (4.8 ± 0.9%; p = 0.002). Additionally, the colon length was significantly shortened compared to control (4.8 ± 0.3 versus 6.1 ± 0.2 cm; p = 0.026). Also further parameters showed a tendency towards more inflammation in VilCre-CMVcaNrf2 mice, such as MPO activity (195.0 ± 24.7 versus 143 ± 35.5 in control mice; p = 0.175) and histological score, which was 5.9 ± 0.5 and 8.2 ± 0.5 respectively (p = 0.067).
Mice overexpressing Nrf2 in the myeloid lineage lost significantly more weight (13.7 ± 1.4 versus 6.4 ± 1.7%; p = 0.006). Histological score of colon sections was 7.4 ± 0.4 in LysMCre-CMVcaNrf2 mice compared to 4.9 ± 0.6 in control mice (p = 0.019), indicating more severe inflammation. However, colon length and MPO activity did not differ between groups.

Conclusion: Our findings show that overexpression of Nrf2 in epithelial cells as well as in myeloid cells leads to a higher susceptibility to DSS induced acute colitis in mice. Further studies aim at investigating whether these effects are due to a reduced antibacterial defense by reactive oxygen species.
The gene encoding the G protein-coupled receptor 68 (GPR68/OGR1) is regulated by TNF, hypoxia, and low pH in human monocytic cells

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Background: The G protein-coupled receptors (GPCR), ovarian cancer G protein coupled receptor 1 (OGR1/GPR68), GPR4 and T-cell death associated gene 8 (TDAG8/GPR65) function as sensors for extracellular protons, leading to activation of adenyl cyclase or phospholipase C. Inflammatory bowel disease (IBD) is associated with acidification of mucosal tissue, and subsequent proinflammatory cytokine production. The cytokine tumor necrosis factor (TNF) is of crucial importance in IBD pathogenesis, and many of its effects are mediated by the transcription factor nuclear factor-κB (NF-κB). Furthermore, tissue hypoxia, which stabilizes the transcription factor hypoxia-inducible factor-1α (HIF-1α), is a feature of active IBD. We studied whether TNF and hypoxia regulate the expression OGR1 and TDAG8, which are expressed in both immune cells and colonic tissue.

Methods/Results: Treatment of the human monocytic cell line MM6 cells with TNF, but none of the other cytokines (IFN-γ, IL-1β, IL-6, TGF-β) tested, led to significant upregulation of OGR1 mRNA, in a dose-dependent manner, as determined by real-time PCR. Macrophagic differentiation of MM6 cells with phorbol myristate acetate (PMA) also led to a significant increase in OGR1 mRNA expression. TNF- and PMA-dependent induction of OGR1 mRNA was confirmed in primary human monocytes. Induction of OGR1 mRNA expression by TNF was reversed by simultaneous treatment of cells with specific NF-κB inhibitors. In agreement with the findings at the mRNA level, GPCR activity in MM6 cells was increased upon treatment with TNF or PMA, only at acidic pH, as quantified by intracellular calcium dyes and label-free detection. In hypoxia, both 0.2% and 2% oxygen conditions enhanced TNF- and PMA-induction of OGR1 mRNA expression. Consistent with the hypoxia effect in MM6 cells, OGR1 expression was elevated in colonic tumors in mice. Two alternative predicted promoter variants ~9 kbp apart, exist for the OGR1 gene in chromosome 14. In silico analysis using MatInspector software and visual inspection revealed several putative DNA-binding sites for NF-κB and HIF-1α within the proximal regions of the OGR1 promoter variants. In addition to TNF and hypoxia, OGR1 mRNA expression was elevated at low pH, suggesting positive feed-forward regulation of OGR1 activity in acidic conditions. No induction of mRNA expression of the related GPCR, TDAG8, was observed upon treatment with TNF or PMA.
**Conclusion:** OGR1 expression is induced in cells of human macrophage lineage and primary human monocytes by TNF and PMA. NF-κB inhibition reverses the induction of OGR1 mRNA expression by TNF. Interestingly, hypoxia, known to cross-talk with the NF-κB pathway, enhances the TNF-mediated induction of OGR1 expression. The stimulation of OGR1 expression by TNF and hypoxia, and subsequently pH-sensing activity, may play a role in IBD pathogenesis.
Functional consequences of G protein-coupled receptor 68 (GPR68/OGR1) overexpression in intestinal epithelial cells

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Introduction: A single layer of epithelial cells creates a physical barrier between the external lumen and the internal milieu of the gastrointestinal tract. During inflammation, which is typically associated with a decrease in local pH, the intestinal barrier can be disrupted. Defects in barrier integrity have been implicated in the pathophysiology of inflammatory bowel disease (IBD). However, the molecular mechanisms mediating the responses to changes in pH are not well understood. G protein-coupled receptor 68 (GPR68), also known as ovarian cancer GPR1 (OGR1), is a proton-sensing receptor, which upon acidification stimulates second messenger signalling, such as intracellular calcium flux, inositol phosphate (IP) formation, and extracellular signal-regulated kinase (ERK) phosphorylation. We have investigated OGR1-mediated signalling pathways in response to lowered pH in intestinal epithelial cells. The colon carcinoma-derived Caco2 cell monolayers can be used as in vitro cell culture models of the human intestinal epithelium.

Methods: The OGR1 cDNA fragment was generated by PCR, and cloned into the pcDNA3.1 vector carrying a neomycin resistance gene. Clonal selection of stably transfected Caco2s was by limiting dilution or cloning cylinders, using the selection agent G418 (400 μg/ml). OGR1-overexpressing clones were selected by positive mRNA/protein expression and immunofluorescence, and those exhibiting strong IP formation upon acidification were chosen for further studies.

Results: The selected Caco2-OGR1 clones showed a typical pH-dependent GPCR response, in both intracellular calcium signalling and label-free assays (EPIC). In addition, they exhibited weak but significant formation of cAMP upon extracellular acidification. When compared to the Caco2 vector control clone, the Caco2-OGR1 clones showed ERK phosphorylation after a pH shift from pH 7.9 to pH 6.8 for 5 minutes, by Western blotting. No pH-dependent phosphorylation remained present after 30 minutes. The Caco2-OGR1 clones exhibited significant serum response factor (SRF) activity at acidic pH but not at alkaline or neutral pH, in dual luciferase assays using SRE-dependent promoter constructs. Consistent with this, mRNA expression of a SRF target gene c-FOS was elevated in parallel. Electric Cell-Substrate Impedance Sensing (ECIS) assays showed an improvement of the barrier function in the Caco2-OGR1 monolayers at acidic pH, but not at neutral or alkaline pH.

Conclusion: We have created Caco2 cell clones stably overexpressing OGR1, and have shown that these serve as valid tools to study OGR1 function and OGR1-mediated signalling. OGR1 overexpression in intestinal epithelial cells enhanced ERK signalling, led to improved barrier function, and increased SRF activity. Unravelling OGR1-dependent signalling may aid our understanding of the pathophysiology of IBD.
Bioequivalence results for a new azathioprine 100 mg tablet

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Introduction: Azathioprine, an imidazolyl derivative of 6-mercaptopurine, is a classical immunosuppressive agent with a high degree of clinical success in a broad range of severe diseases including inflammatory bowel disease. The aim of the presented crossover study was to show bioequivalence between a new azathioprine formulation with a higher strength (100 mg tablet) and the reference medicinal product (50 mg tablet).

Methods: Sixty-six healthy subjects (female:male, 1:1) received a single dose of one new azathioprine 100 mg tablet (test drug) and a single dose of two Imurek® 50 mg tablets (reference drug) in random order and separated by a wash out period. Twenty-one venous blood samples were collected during 24 hours after drug intake for simultaneous determination of azathioprine and 6-mercaptopurine in plasma. Lower limit of quantification of validated liquid chromatography tandem mass spectrometry was 1 ng/ml in plasma for both analytes. Pharmacokinetic and statistical analyses were performed according to the EMA guideline on the investigation of bioequivalence.

Results: For area under the plasma concentration time curve from administration to time of last observed concentration (AUCt) and maximum plasma concentration (Cmax) of both azathioprine and 6-mercaptopurine the 90% confidence intervals for the ratio of the test and reference products were contained very well within the acceptance interval of 80.00 to 125.00%.

Discussion/Conclusion: The results demonstrate bioequivalence of a new azathioprine 100 mg tablet and the reference medicinal product not only for the parent compound but also for the metabolite.

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IL-28 signaling in intestinal epithelial cells leads to a revised mucosal wound healing

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Type III interferones like IL-28 were recently discovered and signal through a unique receptor consisting of the IL28Rα and IL10Rβ subunits which leads to the phosphorylation of STAT1. This receptor has been demonstrated to be highly expressed on epithelial cells. The aim of this study was to determine the functional role of IL28Rα expression on epithelial cells in the pathogenesis of experimental colitis.

Induction of colitis in mice by administration of DSS resulted in a significant upregulation of IL-28 expression in the gut. Treatment of primary intestinal epithelial cells with IL-28 leads to phosphorylation of p38 and STAT1 that are involved in cell cycle progression as well as in wound healing processes. Furthermore, an analysis of the pathogenesis of DSS colitis demonstrated that IL-28Rα KO mice display a significant upregulation of the inflammation of the gut compared to WT mice. Furthermore, intestinal sections from these mice revealed a dramatic downregulation of proliferation of epithelial cells in the gut from KO mice, suggesting a delayed wound healing process. Additional wound healing experiments confirmed our findings regarding the decelerated wound closure after injury of the gut epithelium. Interestingly, topical administration of IL-28 onto the wound induces a better and faster closure of the lesion.

IL-28 directly affects intestinal epithelial cells, activating proliferation and aiding in wound healing in the DSS model of colitis. Thus, treatment with type III interferons may serve as a potential new therapeutic method to protect against chronic intestinal inflammation and promote wound healing processes in the context of colitis.
Endothelin-1 inactivating peptidases and adiponectin in mucosa in children with inflammatory bowel disease (IBD)

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Endothelin-1 (ET-1), when present in excess in the gut mucosa, may play a role in pathophysiology of Crohn’s disease (CD) and ulcerative colitis (UC). Recently, we have described (Regulatory Peptides, 2008) three specific ET-1 inactivating peptidases in rat duodenal and intestinal mucosa that may regulate local concentration of the peptide. Secretion of anti-inflammatory adiponectin is inhibited by increased ET-1 level.

Aim: to show whether ET-1 degrading peptidases are present in human GI mucosa, and whether their activity is diminished in CD and UC resulting in the accumulation of ET-1, mucosal damage and decrease of adiponectin secretion.

Methods: Mucosa and blood samples were taken from children with UC (n = 6), CD (n = 19) and non-IBD controls (n = 12) for the diagnostic examinations and a part of each sample was used for this study. Activity of the ET-1 inactivating peptidases in the mucosal extracts was quantified using 125I-ET-1 as a substrate and expressed in pM of hydrolyzed 125I-ET/min/mg protein. Serum and mucosal adiponectin was assayed with RIA.

Results: Human duodenum and small intestine mucosa contains three highly active ET-1 degrading peptidases: acidic, neutral and alkaline. The enzymes were absent in the large intestine mucosa in IBD and non-IBD controls. Activity of the acidic, neutral and alkaline ET-1 degrading peptidases in the duodenal mucosa from non-IBD children was: 4.52 ± 3.89, 3.07 ± 2.77 and 2.77 ± 2.55 pM/min/mg. In the small intestine mucosa the respective values were: 4.71 ± 1.45, 3.96 ± 1.91 and 4.68 ± 3.48 pM/min/mg. Activity of the peptidases in CD and UC children was not significantly different from the values obtained for non-IBD controls (p > 0.05). Serum adiponectin level was significantly higher in UC (9.21 ± 2.68) and CD children (11.7 ± 4.21) compared to controls (6.25 ± 3.01 µg/ml, p < 0.05). Adiponectin level in the duodenal and small intestine mucosa was similar in IBD patients (20.8 ± 0.84) and in controls (21.8 ± 7.32 ng/mg protein).

Discussion/Conclusion: Activity of the ET-1 degrading peptidases, present in duodenum and small intestine mucosa, is not diminished in UC and CD children. The enzymes may regulate local ET-1 concentration. Whether large intestine mucosa is “defenseless” to an excess of ET-1 remains to be determined. Anti-inflammatory potential of adiponectin in circulation and mucosa is not decreased in IBD.
Infliximab and adalimumab modulate apoptosis of lamina propria lymphocytes in patients with Crohn’s disease in Fas-independent pathway

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Introduction: TNF-α inhibitors (infliximab – IFX, adalimumab – ADA) have a great potential of reducing inflammatory infiltration in the gut mucosa in patients with inflammatory bowel diseases. One of postulated mechanisms of anti-TNF agents action is induction of apoptosis of lamina propria lymphocytes (LPL). The aim of the study was to evaluate the influence of TNF-α inhibitors on Fas-mediated apoptosis of LPL in patients with Crohn’s disease (CD).

Methods: 35 patients with active, not responding for standard therapy, CD were enrolled in the study. Colonoscopy was performed before starting biological therapy and after induction phase of anti-TNF treatment. Activity of CD was assessed calculating Crohn’s Disease Activity Index (CDAI) and Simple Endoscopic Score for Crohn’s Disease (SES-CD). Tissue sampling was performed during each endoscopy for histological analysis from involved areas of the colon. Expression of active caspase 3 and Fas in LPL was assessed using immunocytochemistry. Data were analyzed statistically.

Results: Among patients who responded to the therapy (CDAI < 150 and SES-CD ≤ 5) we observed a strong increase in active caspase 3 expression in LPL, in contrast to those patients without response. The potential of inducing LPL apoptosis was comparable between IFX and ADA. There was no significant influence of anti-TNF treatment on Fas expression in LPL. There was no correlation between expression of active caspase 3 and Fas in LPL.

Discussion/Conclusion: Induction of LPL apoptosis is one of the major pathways in which IFX and ADA realize their therapeutic efficacy. This phenomenon is independent on Fas expression in these cells.
Influence of anti-TNF agents on mucosal inflammation in patients with Crohn’s disease – Preliminary report

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Introduction: Anti-TNF agents have a strong potential of healing the mucosa in patients with Crohn’s disease (CD), however direct mechanisms of that phenomenon are unknown. The aim of the study was to assess histological changes in mucosa of patients with CD after treatment with anti-TNF antibodies and its relation to mucosal healing effect observed in endoscopy.

Methods: 35 patients with CD were enrolled in the study. All patients were treated with TNF-alpha inhibitors (infliximab or adalimumab). Colonoscopy with tissue sampling was performed before and after induction phase of anti-TNF treatment. Histological activity was assessed using microscopic classification proposed by D’Haens and colleagues, endoscopic activity was assessed calculating Simple Endoscopic Score for Crohn’s Disease (SES-CD). Response to the induction therapy was defined as Crohn’s Disease Activity Index < 150 and SES-CD ≤ 5 pts.

Results: 21 patients responded to the therapy. In this group we observed a significant reduction of microscopic activity of inflammation in mucosal biopsies (p < 0.04). Among 33% patients complete epithelial repair was noted, complete normalization of granulocytes and mononuclear cells infiltration in lamina propria were observed in 33.3% and 14.2% of patients, respectively. Disappearance of erosions/ulcerations was observed in all patients who had had these microscopical findings before treatment. SES-CD correlated significantly with microscopic activity before and after treatment (p < 0.05).

Discussion/Conclusion: In all responders we observed the mucosal healing effect and it correlated with microscopical healing. Microscopical healing process consisted first of all of epithelial repair, decrease of leukocyte infiltration and disappearance of erosions/ulcerations in the mucosa.
Chronic hepatitis C, inflammatory bowel disease and interferon therapy

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Aims: Investigating the exacerbation of preexisting or de novo induction of ulcerative colitis (UC) which has been reported during interferon (IFN) therapy.

Methods: We evaluated data from 102 patients with hepatitis C (HCV) in order to detect whether IFN may cause new onset or exacerbations of underlying UC.

Results: Two patients were identified; one was diagnosed with HCV during the initial presentation of UC. The patient was treated with mesalazine, leading to remission. Afterwards IFN and ribavirin combination was commenced. The reactivation of UC was not observed during therapy but, despite three months of therapy serum viral load was in detectable levels; hence therapy was discontinued. The other patient developed UC two weeks after IFN therapy. All symptoms of UC were controlled by mesalazine however, the patient refused to proceed with the same therapy due to potential side effects.

Conclusion: Reactivation or new onset of UC has been reported in HCV patients treated by IFN. But, these are anecdotal reports and limited to only single case studies. On the other hand, in large population based studies, reactivation of UC was not observed in patients undergoing IFN therapy for HCV. To date, many HCV patients have been treated with IFN but, exacerbation of UC has been rarely observed. Therefore, it is difficult to consider IFN as a strong predisposing factor for UC exacerbation. Our data along with existing literature, suggests that commencing IFN therapy in patients with concomitant UC and HCV, while UC is in remission, is plausible.
The increasing incidence of inflammatory bowel diseases (IBD) in Nile Delta area of Egypt

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Introduction: Endoscopists have reported an increasing incidence of inflammatory bowel diseases (IBD) and colorectal cancer (CRC). This may be explained by an increasing index of suspicion and the availability of endoscopy. Population-based studies are lacking. The aim of our study was to retrospectively evaluate final diagnosis in patients subjected to colonoscopy in Tanta University Hospital and affiliated hospitals at the Nile delta area of Egypt, which is one of the most densely populated regions in the country.

Methods: This study was done at the endoscopy units of Tanta University Hospital and affiliated hospitals from June 2009 to June 2010. A total of 864 patients presented with different indications for colonoscopy. All findings were recorded, analyzed, and discussed.

Results: Colonoscopy revealed a diagnosis of ulcerative colitis (UC) in 22%, haemorrhoids in 18%, CRC in 15%, benign colorectal polyps in 9%, Crohn’s disease (CD) in 3%, diverticulosis in 2%, and anal fissures in 2% of patients. No organic colonic disease was found in 28% of patients. Complications occurred in less than 1% of the cases.

Discussion/Conclusion: In Egyptian patients subjected to colonoscopy, the most frequent diagnoses were UC, followed by haemorrhoids, CRC, benign polyps, and CD. This may represent an increasing incidence of UC and CRC. Colonoscopy was safe and few complications were recorded. Prospective population-based studies are needed in order to measure the incidence, prevalence, and risk factors of various diseases of the colon in Egypt.
Arthritis as a presenting symptom of subclinical Crohn’s disease

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Crohn's disease (CD) is an immune-mediated, chronic inflammatory disease that can affect any part of the gastrointestinal tract from the mouth to the anus. Rheumatic, mucocutaneous, ophthalmologic, thromboembolic manifestations, cholelithiasis, nephrolithiasis, extraintestinal findings, such as around 40–50% during the course of the disease is rarely seen as the first signs may appear. Several cases of rheumatological extraintestinal manifestations that appeared concomitantly with CD have been reported. Herein we describe a patient with monoarthritis that led to the diagnosis of asymptomatic CD.

A 47-year-old female was consulted to our clinic for dyspeptic symptoms. She had been referred to rheumatology clinic for evaluation of recurrent episodes of joint pain and swelling involving the left knee during last 6 months. Laboratory investigation showed an elevated erythrosedimentation rate of 52 mm in the 1st hour, CRP of 7.43 (0–0.8) mg/l, hypochromic microcytic anemia with hemoglobin level of 11.6 g/dl, and low serum iron levels. The rest of the blood count and serum chemistry studies were all within normal ranges. Extensive investigations including serological tests for autoimmune and infectious diseases, human leukocyte antigen B27, chest, and sacroiliac joint radiographs were all negative. Despite the non-steroid anti inflammatory drug treatment, CRP and erythrosedimentation rate increased gradually over the last three months. Her upper gastrointestinal endoscopy revealed pangastritis. Colonoscopic examination was performed with suspicious of inflammatory bowel disease. It disclosed hyperemic ulcerated terminal ileum. Biopsies revealed a marked inflammatory infiltrate of the mucosa and submucosa compatible with CD. Therapy with 3 g/day of 5-ASA was started, both joint pain and laboratory findings were improved in follow up.

Arthritis occurs in 9–50% of patients in inflammatory bowel disease. Peripheral arthritis may be acute and remitting (type I) or a chronic problem with frequent relapses (type II). In type I arthropathy, the peripheral arthritis usually occurs early in the course of the bowel disease, and doesn’t result in joint deformities. The knee is most commonly affected. Joint symptoms may occur prior to the onset of symptoms suggestive of bowel disease. Effective treatment of the underlying IBD is often helpful in controlling the peripheral arthritis. Sulfasalazine and immunomodulatory agents are effective controlling both in bowel and joint symptoms. Reviewing the literature there is only one case of CD presenting with arthritis and without evidence of any bowel symptoms.

Symptoms and signs such as anemia, weight loss, elevated sedimentation rate, and CRP levels that can be erroneously attributed to the rheumatological diseases. Undifferentiated arthritis may be a precursor of subclinical gut inflammation and CD should be kept in mind in differential diagnosis to avoid diagnostic delay.
Nutrition status of Crohn’s disease patients and risk factors of malnutrition: A prospective study

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Introduction: Malnutrition is common in patients with inflammatory bowel disease (IBD), especially in active Crohn's disease (CD). Several studies have documented weight loss in 70–80% of hospitalized IBD patients and in 20–40% of outpatients with CD. The aim of our study is to evaluate the nutrition status of CD patients and to precise risk factors of malnutrition.

Patients and methods: We included prospectively all patients with Crohn’s disease (hospitalized and outpatients). For each patient we evaluated the nutrition status by the body mass index (BMI). We considered malnutrition if the BMI is < 18.5 kg/m². The malnutrition was classified as major if BMI < 15, severe: 15 < BMI < 17 and moderate if 17 < BMI < 18.5). The following parameters were analysed: age, sex, duration and location of Crohn's disease, smoking status, haemoglobin, albumin, cholesterol, triglycerides, calcium and phosphor).

Results: We included 77 patients, 40 women and 37 men with a mean age of 35 years. 45.5% of patient’s were hospitalized and 54.5% were outpatients. The mean duration of the disease was of 65 months (1–300). The location was respectively colic, ileocolic and ileal in 42.9%, 39% and 18.1% des patients. Only 16.9% of patients had an ileal resection. At inclusion, 32% were treated by azathioprine. The Crohn’s disease was active in 32.5% of cases. A malnutrition was diagnosed in 11patients. It was moderated in 6 cases, severe in 3 cases and major in 2. 9 of these patients were hospitalized, 8 had active disease and none of them was treated by azathioprine. The risk factors of malnutrition were the hospitalization (p = 0.009), the active disease (p = 0.002) and the no using of azathioprine (p = 0.003). A positive correlation was found between the BMI and triglycerides level (r = 0.3, p = 0.006), cholesterol concentration (r = 0.22, p = 0.048), albumin (r = 0.28, p = 0.01) and haemoglobin (r = 0.23, p = 0.043).

Conclusion: A malnutrition was observed in 14.3% of Crohn’s disease patients. It was moderated in half of them. Hospitalization, active disease and the no use of azathioprine were the risk factors of this nutrition status.
Bone loss in Crohn’s disease and risk factors

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Introduction: Patients with Crohn’s disease (CD) are at greater risk of developing osteoporosis and osteopenia than healthy controls. The aim of the study was to determine the prevalence and risk factors of osteoporosis in patients with CD.

Methods: CD patients followed in our department were prospectively recruited. The following parameters were analysed: age, sex, Crohn’s Disease Activity Index, duration and extent of Crohn's disease, smoking status, corticosteroid treatment, immunosuppressive drugs, plasma homocysteine, folate and vitamin B12 concentration. Dual-energy X ray absorptiometry measurements of bone mineral density (BMD) were obtained at the femoral neck and at the lumbar spine.

Results: Fifty-five patients were consecutively included: 26 men and 29 women, with a mean age of 36 years (15–60 years). The Crohn’s location was ileal in 9.1%, ileo-colic in 34% and colic in 36.4%. Approximately, one half of patients took azathioprine. Osteoporosis was found in 32.5% and osteopenia in 47.5% of patients. Median body mass index (BMI) was lower in patients with osteoporosis than in those without osteoporosis (17.67 versus 21.8, p = 0.001). The mean concentration of homocysteinemia was 13.41 µmol/l. An hyperhocystenemia (> 15 µmol/l) was correlated with osteoporosis measured at femoral neck. Neither disease duration nor steroid use were associated with bone loss.

Conclusion: Malnutrition seems to be a major risk factor of bone loss in patients with CD. It should be taken into consideration when planning treatment programs.
Hyperhomocysteinemia in Crohn’s disease: Frequency and risk factors

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Background and aims: A high prevalence (52%) of hyperhomocysteinemia is observed in Crohn’s disease (CD). The role of hyperhomocysteinemia (HHC) and its determinants in CD remain uncertain. This study was aimed to determine the prevalence of HHC and its main risk factors in Tunisian patients with CD.

Methods: CD patients followed in our department and age and sex matched healthy subjects were prospectively recruited. Patients with kidney failure or drugs supposed, to interfere with homocysteine metabolism (folates, vitamin B₁₂, methotrexate) were excluded from the study. The following parameters were analysed: age, sex, clinical activity indexes (CDAI), location of the disease, smoking, plasma homocysteine concentration, folates and vitamin B₁₂ and C-reactive protein and creatinine levels. Logistic regression models were applied to identify factors associated with HHC in CD patients.

Results: 89 patients with CD and 103 age- and sex-matched healthy subjects. Plasma homocysteine was higher (13.69 ± 4.84 μmol/l vs. 10.77 ± 2.80 μmol/l; p < 0.01) in CD’s patients versus healthy subjects. An hyperhomocysteinemia was more frequent (31.5% vs. 7.8%; p < 0.001) in patients compared with controls. The association between HHC and CD persisted after adjustment for smoking, body mass index and serum folate, vitamin B₁₂, creatinine and C-reactive protein. In patients with CD, multivariate analysis showed that HHC was positively associated with age: 1.14 (1.06–1.24); p < 0.001], active disease [7.54 (1.15–49.3); p = 0.03], disease duration > 2 years [8.69 (1.53–49.3); p = 0.02] and inversely related to plasma folate [0.64 (0.48–0.84); p = 0.002] and vitamin B₁₂ (0.993 (0.987–0.999); p = 0.02) concentration.

Conclusion: HHC is common in patients with CD and is related to B vitamins deficit, as well as disease activity and duration. Further studies should test the effect of correction of HHC by vitamin B supplementation on progression and complications of CD.
Cutaneous manifestations in inflammatory bowel diseases: Results of a cross-sectional study

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Introduction: Skin manifestations are not uncommon in inflammatory bowel disease (IBD). However they should be divided into specific cutaneous signs, reactive lesions, and manifestations due to malabsorption or drug therapy and associated dermatosis.

Aim: To evaluate the frequency and the subtypes of cutaneous manifestations in Crohn's disease (CD) and ulcerative colitis (UC).

Patients and methods: A cross-sectional study including all patients with an IBD who presented to the Department of Gastroenterology. A systematic examination of skin, mucosa, hair and nails was performed. Cutaneous manifestations were classified in 4 subtypes: specific cutaneous signs, reactive lesions, manifestations due to malabsorption or drug therapy and associated dermatosis.

Results: One hundred-ninety-three (193) patients were examined (102 man and 91 woman) of a mean age 39.5 years (19 61). Among these patients, 79.8% had CD and 21.2% had UC. Specific cutaneous lesions were observed in 81 cases (52.6%), mainly fistulas, infiltrated deep rhagades and ulcers. Reactive lesions were noted in 7.25% (pyoderma gangrenosum, erythema nodosum and stomatitis aphtosis). Cutaneous manifestations due to malabsorption were noted in 34.2% (ichthyosiform state, eczema, glossitis, loss hair) and those due to treatment in 18.13% (acneiform dermatitis, rosacea, drug eruption). Associations with other dermatosis were observed in 7.77% of the patients (psoriasis, lichen planus, alopecia areata and neurofibromatosis). A high frequency of mycoses was observed (24.5%).

Conclusion: Cutaneous manifestations of IBD were diverse and frequent in our study. Specific lesions were the most common but those due to malabsorption were also frequently observed in our study. Associated dermatoses such as psoriasis were observed and have a still questionable connection with IBD.
Fecal calprotectin is a predictive marker of relapse in Crohn's disease involving the colon: A prospective study

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Objectives: Fecal calprotectin (FC) is a relatively new marker of intestinal inflammation. Recently, many studies have extended its role in predicting relapse of quiescent inflammatory bowel disease (IBD), but the reported results have been inconsistent. The aim of this study was to prospectively evaluate the role of fecal calprotectin as a predictive marker for 1-year follow-up in patients with inactive Crohn's disease.

Methods: Crohn's disease patients in clinical remission were consecutively included providing at the beginning of the study a single stool sample as well as a blood sample and regularly followed-up for 12 months. Fecal calprotectin level was measured using a commercially available enzyme-linked immunoassay.

Results: Fifty-three patients were included. 10 (18.9%) developed clinical relapse during the 12-month follow-up period. Median fecal calprotectin level was significantly higher in relapse group patients compared with non-relapse group (380.5 vs. 155 µg/g, p < 0.001). A cutoff value of 340 µg/g fecal calprotectin gave sensitivity of 80% and specificity of 90.7% in predicting clinical relapse. Fecal calprotectin level greater that 340 µg/g gave an 18-fold higher risk to develop relapse (log rank p < 0.001) and was found to be an independent predictive factor of relapse (p = 0.02).

Conclusion: Fecal calprotectin seems to be a reliable marker of relapse in quiescent Crohn's disease patients.
Exogenous heat shock protein gp96 ameliorates CD4+CD62L+ T cell-mediated transfer colitis in a mouse model

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Background: Transfer of naive CD4+CD62L+ T cells into immunodeficient mice is a well-characterized model mimicking the immunological mechanisms responsible for induction and regulation IBD in humans. Mice develop clinical signs of inflammation three weeks after transfer, accompanied with a specific loss of gp96 protein in mature F4/80 positive IMACs in the inflamed mucosa. Previous studies revealed a comparable specific loss of gp96 protein in intestinal macrophages (IMACs) of Crohn's disease patients. Since exogenous gp96 might be able to prime T-cells in vivo, we assessed the ability of gp96 treatment to ameliorate transfer colitis in mice.

Methods: Transfer colitis was induced in immunodeficient CB17 SCID mice. Animals were treated with either gp96, murine serum albumin (MSA) or sterile PBS twice, 1 and 8 days after transfer. Course and extent of intestinal inflammation were determined by body and spleen weight, colonoscopy, histology, cytokine expression and activity of myeloperoxidase.

Results: While healthy controls gained weight during the experiment (8.1% ± 1.9), untreated colitis animals significantly lost weight (-6.8% ± 3.0). Animals treated with MSA or gp96 showed a diminished relative loss of body weight (-2.4% ± 2.3, and -0.4% ± 2.0, respectively). Endoscopic analysis of inflammation (MEICS score) revealed strong signs of inflammation in untreated colitis animals (3.8 ± 0.8) compared to healthy controls (0.3 ± 0.3). Also MSA treated animals showed strong changes (2.8 ± 0.1), while gp96 treatment restored macroscopic signs of inflammation (1.5 ± 0.8). Strong histological signs of inflammation were seen in untreated animals suffering from colitis (3.4 ± 0.2), compared to healthy controls (0.0 ± 0.0). Treatment gp96 resulted in ameliorated histological signs of inflammation (2.2 ± 0.3.). Relative spleen weight of untreated colitis animals was found to be significantly increased (8.1 ± 4.0) compared to healthy controls (1.5 ± 0.8). While treatment with MSA even increased the effect (7.3 ± 1.3), gp96 treatment restored relative spleen weight (2.6 ± 0.8). Gene expression profiles of colonic specimen revealed lower levels of TNFα (0.7-fold) and IFNγ (0.5-fold) in gp96 treated animals than in untreated colitis animals. Myeloperoxidase activity was used as a quantitative assessment of neutrophil infiltration. While healthy controls showed the lowest activity (0.05 ± 0.00), it was increased in untreated (0.13 ± 0.04) and MSA treated (0.09 ± 0.02) colitis animals. gp96 treated animals (0.04 ± 0.01) showed activity comparable to healthy controls.

Conclusion: Our observations show that gp96 treatment ameliorates experimental transfer colitis. gp96 might affect naive T cells epigenetically, exerting a memory effect. A deeper understanding of the underlying regulatory mechanisms will essentially contribute to reveal new therapeutic treatment options.
Colitis-associated colon cancer is controlled by the IL-6 level and the transcription factor NFATc2

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**Introduction:** NFATc2 (Nuclear factor of activated T-cells) belongs to a transcription factor family regulating Th2 activation, cytokine production, the cell cycle and the cell growth implying a role in cancer development. Furthermore NFATc2 has an influence on the pro-tumorigenic cytokine IL-6. Therefore we examined the role of NFATc2 in the establishment of colon cancer mediated by IL-6 expression.

**Methods:** NFATc2ko mice were treated with AOM/DSS to induce colon cancer. Miniendoscopic analysis has been done to monitor the number and size of the tumors. Sections of normal and tumor tissue were stained with different antibodies. Cytokine measurements were done by ELISA and RT-PCR.

**Results:** In our experimental colitis-associated cancer model NFATc2ko mice do not develop colorectal tumors, in contrast to wildtype. The protection of colonic tumor progression was associated with increased apoptosis rate in the lamina propria of NFATc2ko mice and significantly increased levels of IL-6 in the serum and the colon. Further analyzing IL-6 involvement in the colon cancer model, the application of hyper IL-6, a fusion protein of IL-6 and soluble IL-6-receptor, to the NFATc2ko mice abrogated the protective effect and restored the tumor development approving the pro-tumorigenic effect of IL-6.

**Conclusion:** NFATc2 is a key regulator for the establishment of colitis-associated cancer as the NFATc2ko mice did not develop tumors in the experimental model. Besides NFATc2 is able to control IL-6 levels which are important for the cancerogenesis.
Measurement of iohexol in serum as an alternative to urine test for assessment of intestinal permeability in patients with inflammatory bowel diseases

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**Aim:** To study the intestinal permeability (IP) in patients with inflammatory bowel diseases (IBD) – Crohn’s disease (CD) and ulcerative colitis (UC) by measuring serum and urine levels of water-soluble contrast medium iohexol and to compare the reliability of serum versus urine iohexol levels as an IP disease marker.

**Methods:** Fifty-eight patients with active IBD (32 with CD and 26 with UC) and 25 healthy controls consented to participate in the study. IP was assessed by the iohexol, which was administered orally (25 ml, 350 mg/ml) 2 hours after breakfast. Three and six hours later serum (SIC mg/l) and urine (UIC g/mol) iohexol concentrations were determined by a validated HPLC-UV technique.

**Results:** In the CD group, the mean values of SIC at 3 h (2.95 ± 2.11 mg/l) and at 6 h after ingestion (2.63 ± 2.18 mg/l) were significantly higher than those in the control group (1.25 ± 1.40 mg/l and 1.11 ± 1.10 mg/l, respectively). UIC were also higher in patients compared to the controls, but the difference was significant only for the mean UIC values at 6 h (36.92 ± 27.68 g/mol vs. 14.18 ± 7.78 g/mol). In UC group, the mean serum levels (1.57 ± 1.55 mg/l and 2.49 ± 2.80 mg/l) and urine recovery of iohexol in two studied periods (9.86 ± 9.26 g/mol and 27.76 ± 25.18 g/mol, respectively) were higher than those for healthy controls, but the differences were significant only at 6 h for both parameters.

**Conclusion:** The water-soluble contrast medium iohexol is a suitable marker for assessing the IP in patients with IBD. Measurement of a single serum sample of iohexol 6 h following its oral administration makes the proposed permeability test more convenient and provides a possibility for the assessment of altered barrier function in both small and large intestine.
Duration of intravenous steroid therapy in patients with severe attack of ulcerative colitis

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Introduction: Intravenous glucocorticosteroid therapy is the main method for management of severe attack of ulcerative colitis. The optimal duration of intravenous steroid therapy remains controversial, varying from 3 days to 4 weeks. The present study was performed to identify a deadline when resistance to steroids can be determined, and to define early clinical, laboratory and endoscopic predictors of inefficacy of intravenous steroids.

Methods: A retrospective analysis included 91 patient admitted to state scientific center for coloproctology with severe attack of ulcerative colitis (mean age 37.9 years [range: 17–69], 58% males). Extensive colitis was present in 75% of subjects. All patients received 2 mg/kg/day intravenous prednisolone, antibiotics and fluid maintenance.

Results: A satisfactory response (SR) – less than 4 stools/day without visible blood in feces – was achieved in sixty-one patient out of 91 (67%). Mean time for establishment of SR was 10.5 days (3–22). Ninety-four percent of patients without SR on Day 15 of intravenous therapy underwent colectomy. Parameters, distinguishing in patients with and without SR, were entered in multivariate procedure to determine predictors of colectomy after ineffective steroid therapy in severe attacks of ulcerative colitis. Hemorrhagic stool frequency more than 5 times/day on day 6 of intravenous steroid therapy together with serum albumin level lower than 35 g/l predicted colectomy with 83% sensitivity and 95% specificity.

Discussion/Conclusion: Maximal duration of steroid intravenous monotherapy should not exceed 14 days. If on day 6 of intravenous steroid treatment hemorrhagic stool frequency exceeds 5 times/day and serum albumin level if lower than 35 g/l, immunosuppressors, anticytokine therapy or colectomy are indicated.
Immunohistochemical assessment of MMP-7 expression in IBD

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Introduction: The extracellular matrix (ECM) is a specific matrix participating in the migration, cell adhesion, differentiation, and inter-cell interactions. Remodeling of the ECM is important in the development of many disease processes. A key mechanism is metalloproteinases activity (MMPs) which has the capacity to its degradation and remodeling. One of the proteins belonging to the MMPs is matrix metalloproteinase 7 (MMP-7) whose overexpression was observed in inflammatory and neoplastic processes. Therefore, the aim of this study was to analyze the expression of MMP-7 matrilysin in inflammatory bowel disease.

Material and Method: The study group consisted of 41 patients diagnosed with ulcerative colitis and 10 with Crohn's disease. The biopsy slices were used as the study material in which the expression of MMP-7 protein was determined by immunohistochemical method with the use of monoclonal antibodies and standard immunoperoxidase technique. The staining reaction in a 4-point scale was assessed as absent, weak, medium and strong.

Results: The expression of MMP-7 protein in normal epithelial cells and inflammatory cells was observed. In patients with ulcerative colitis in epithelial cells, the reaction was absent in 54.9%, weak in 29% and medium in 16.1% of cases, while in patients with Crohn's disease the expression was defined as weak in 50%, medium in 40% and strong in 10% of cases. The expression of MMP-7 was higher in inflammatory cells than in epithelial cells of patients with ulcerative colitis that was shown as absent in 6.4% of cases, weak in 35.5%, medium in 32.3%, and strong in 25.8% of cases. In the cases of Crohn's disease it was weak at 20%, medium in 20% and strong in 60%. Statistical analysis showed that increased expression of MMP-7 protein in epithelial cells in patients with ulcerative colitis was associated also with its growth in inflammatory cells (p < 0.000). Moreover, the overexpression of MMP-7 in epithelial cells in patients with Crohn's disease was found to correlate with the location of the disease in the rectum (p < 0.000).

Conclusion: The increased expression of MMP-7 protein appears to be an important factor in the pathogenesis of nonspecific inflammatory bowel diseases.
Can we use fecal calprotectin in the diagnosis of ulcerative colitis among Egyptian patients?

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Introduction: Calprotectin is a promising tool in diagnosis of active inflammatory bowel disease (IBD). The aim of this work was to compare fecal calprotectin in patients known to have active ulcerative colitis with normal healthy controls among Egyptians.

Methods: Twenty-one patients with active disease were studied, 7 males and 14 females. Ten healthy controls (8 females and 2 males). Patients underwent clinical evaluation, determination of blood erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and fecal calprotectin. Colonoscopy was done to confirm diagnosis, estimate disease extent and obtain colonoscopic biopsy specimens for histological grading of activity. An overall scoring of disease activity was done using the Mayo score.

Results: Fecal calprotectin was significantly elevated among patients [mean: 12.6 μg/gm stools in comparison to controls (9.4 μg/gm stools, (p 0.01). At a cut off of 10.3 μm/gm stools it has a sensitivity of 86%, specificity of 70% p 0.004, positive predictive value of 86% and a negative predictive value of 70%. No correlation was found between fecal calprotectin and ESR, histopathology and Mayo score.

Discussion/Conclusion: Fecal calprotectin is a good test in differentiating Egyptian patients with ulcerative colitis from healthy controls. Thus, its use as a screening test may be helpful in the selection of cases for endoscopic examination. It lacks specific correlation with the severity of ulcerative colitis. This leaves endoscopy and histopathological examinations as the main diagnostic tools. Larger scale studies on Egyptian patients are strongly recommended.
High-dose cyclophosphamide and autologous hematopoietic cell transplantation for inflammatory bowel disease – A single center experience

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Background: Despite recent advances in immunosuppressive therapy, up to 10% of patients with severe chronic inflammatory bowel disease remain refractory to conventional treatment. Recent studies show that immune ablation by high-dose immunosuppression and autologous peripheral blood hematopoietic cell transplantation (aPBHCT) may efficiently induce long-lasting clinical remission in autoimmune disease.

Methods: We conducted a phase I/II single center trial of a two step approach of aPBHCT in 13 patients, (3 female, 10 male; age median 36 years) refractory to conventional treatment. Underlying diseases were Crohn’s disease (CD) in 11 patients and ulcerative colitis (UC) in 2 patients. Step 1 included collection of CD34+ selected hematopoietic stem cells (Clinimacs® Cell Separating System, Miltenyi) after mobilization with cyclophosphamide (CTX, 2 x 2 g/m²) and granulocyte-colony stimulating factor (G-CSF). Step 2 contained a conditioning regime with high-dose CTX (50 mg/kg BW day -5 to -2) followed by aPBHCT. Initially, patients already in remission after step 1 were observed without aPBHCT.

Results: Both UC patients showed only transient remission after step 1. They relapsed early, needing proctocolectomy 2 and 4 months after mobilization and thus aPBHCT was not indicated. In contrast, step 1 resulted in good clinical and endoscopic improvement up to 20 months in 10 out of 11 CD patients. Of those, two relapsed severely after 6 months, underwent surgery and were well managed with conventional immunosuppression and autologous peripheral blood hematopoietic cell transplantation (aPBHCT) may efficiently induce long-lasting clinical remission in autoimmune disease.

Conclusion: Immune ablation by CTX followed by aPBHCT is feasible, safe and effective in treating patients with refractory CD and should be further evaluated in randomized controlled trials. For UC it showed no benefit. We propose that aPBHCT should be performed early because mobilization therapy alone is not adequate to prevent early relapses. Furthermore maintenance with low dose immunosuppressive therapy should be initiated early following aPBHCT to stabilize clinical remission.
Non-invasive quantification of volatile metabolites in breath: A potential indicator of inflammatory bowel diseases activity

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Introduction: Inflammatory bowel diseases (IBD) result in localized oxidative stress in the intestinal wall causing degradation of cellular membranes by lipid peroxidation. Volatile organic compounds produced by this process are transported via blood stream and appear in the exhaled breath. The objective was to identify which volatile breath metabolites can serve as biomarkers of IBD disease activity.

Methods: Consecutive IBD patients attending our out-patient clinic were included. Selected ion flow tube mass spectrometry (SIFT-MS) was used to quantify concentrations of acetone, isoprene, propanal, pentane, acetic acid, propanoic acid and butyric acid in breath in real time. Each patient provided three consecutive exhalations; the mean concentration of metabolites was then calculated from the ratios of product ion count rates to the precursor ion count rates. The association between concentrations of identified metabolites and patients’ disease activity was studied.

Results: 40 IBD patients, 16 Crohn’s disease (CD) and 24 ulcerative colitis (UC), were enrolled. Of them 20 were in remission and 20 had active disease. Important differences between subgroups of patients are presented in Table 1.

Discussion/Conclusion: Breath testing using SIFT-MS was found to be feasible in clinical setting in this pilot study. Significant differences observed in several metabolites indicate that point-of-care breath analysis has a potential to provide information about disease activity.

Table 1: Concentrations in parts per billion by volume of three volatile metabolites measured in breath and corrected for inhaled background. For each compound and group mean ± S.D and the range from minimum to maximum are given.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>CD patients</th>
<th>UC patients</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane</td>
<td>104 ± 57*</td>
<td>64 ± 52</td>
<td>46 ± 16</td>
</tr>
<tr>
<td></td>
<td>(41–208)</td>
<td>(16–263)</td>
<td>(16–70)</td>
</tr>
<tr>
<td>Acetone</td>
<td>1870 ± 1221*</td>
<td>453 ± 234</td>
<td>262 ± 91</td>
</tr>
<tr>
<td></td>
<td>(656–3534)</td>
<td>(116–1059)</td>
<td>(122–422)</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>74 ± 30*</td>
<td>67 ± 37*</td>
<td>39 ± 12</td>
</tr>
</tbody>
</table>

*p < 0.05
Bid protein expression in ulcerative colitis

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Introduction: Bid belongs to the family of proteins that induces apoptosis. Its cleavage to terminal form tBid, helps proapoptotic proteins penetrate into the mitochondrial membrane and release cytochrome c, which leads to programmed cell death. Moreover, Bid protein by BH3 domain, may induce apoptosis itself. The role of apoptosis disorder is very well known in the carcinogenesis. Therefore the purpose of this study was to assess the protein expression in ulcerative colitis which tends a progression to colorectal cancer.

Methods: The study was performed by using archived material of 35 patients with ulcerative colitis. The expression levels of Bid was identified by immunohistochemistry.

Results: Bid protein expression was observed in the cytoplasm of normal and dysplastic epithelial cells and were classified as absent or present. In 82.8% (29/35) of patients with ulcerative colitis, lack of expression of Bid protein in dysplastic cells were observed, while a positive expression was present in only 17.2% (6/35) patients. Inverse relationship was observed in these patients in the case of Bid protein expression in normal epithelial cells. Bid expression those cells was not detected in the 22.8% (8/35) cases, whereas it was present in 77.2% (27/35) cases.

Conclusion: Apoptosis of normal intestinal epithelial cells in ulcerative colitis protects against transformation in abnormal dysplastic cells. It can be attested the high level of Bid protein in these cells. Although this process has an important role in the pathogenesis of this disease, because of it does not allow for the healing of the intestinal mucosa. In the dysplastic cells showed a decrease in Bid protein expression which may indicate to inhibition of apoptosis in these cells and their excessive proliferation. It is therefore an important factor that allows normal cells to escape control and transform in dysplastic cells. The mechanism of pathogenesis of ulcerative colitis is poorly understood and the assessment of apoptosis in this disease may help explain this process.
The importance of centralization of biological treatment of IBD patients

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Introduction: In the Czech Republic, there are centres for biological treatment (CBT) for gastroenterology, rheumatology and dermatology. For gastroenterology – i.e. the treatment of inflammatory bowel diseases (IBD) – biological treatment (BT) includes infliximab and adalimumab. Since 2006, there are 26 CBTs in the Czech Republic with more than 1000 patients. 158 patients with IBD have undergone BT at the University Hospital in Olomouc.

Methods: 41 patients with IBD (29 with Crohn’s disease, 12 with ulcerative colitis) underwent BT before the establishment of CBT and continue to undergo BT in 2011. We have examined these patients’ satisfaction with application of BT before and after the CBT establishment using a questionnaire survey. The questionnaire featured 9 questions and patient’s satisfaction was graded at the scale 0–5. The questions referred to doctors’ care, nurses’ care, monitoring of adverse effects, providing information on BT, support from the auxiliary staff, waiting time, informed consent, recommendations and overall satisfaction. The quantitative differences were evaluated by CHI quadrate test and qualitative differences by Student t-test.

Results: Statistically non-significant difference (p = 0.87) before and after the establishment of CBT was only found in evaluation of the level of doctors’ care. In all other monitored items showed statistically significant difference – nurses’ care (p < 0.05), monitoring of adverse effects (p < 0.05), providing information on BT (p < 0.05), support from auxiliary staff (p < 0.05), waiting time (p < 0.05), informed consent (p < 0.05), recommendation and overall satisfaction (p < 0.05).

Discussion/Conclusion: The establishing of CBT for IBD patients in majority of cases has significantly improved the standard of the medical care for IBD patients.
The value of capsule endoscopy in the evaluation of the effectiveness of the treatment of patients with Crohn's disease

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Introduction: Capsule endoscopy (CE) has proven to be extremely useful in diagnosing Crohn's disease (CD) in patients with inconclusive findings from ileocolonoscopy and x-ray-based studies. The utility of CE in monitoring CD and in guiding therapy has also been proposed.

Aim: To explore the effectiveness of CE in monitoring CD and in guiding therapy patients.

Methods: EC was performed in 17 patients with CD in the acute stage. All patients fulfilled the ileocolonoscopy and the X-ray examination of the small intestine to prevent strictures and fistulas. In the EC took into account the number of ulcers, other inflammatory changes (erythema, edema) and their length. Treatment was carried out with the use of budesonide according to the treating physician. After achieving remission (after 30–40 days of treatment) all patients underwent a second CE.

Results: The procedure was performed in all patients. There weren't any complications and capsule retention. All patients were identified after treatment ulcers, but their number has decreased significantly from (6.9 ± 2.1) to (4.3 ± 1.3). The severity of erythema and edema and their length were decreased.

Discussion/Conclusion: CE – an effective method in monitoring CD and in guiding therapy. We found no correlation to improve the clinical picture of diseases and conditions of the mucous membrane of the small intestine, which is consistent with findings of other researchers.
The value of capsule endoscopy in the diagnosis of Crohn’s disease

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Introduction: Capsule endoscopy (CE) has proven to be extremely useful in diagnosing Crohn's disease (CD) in patients with inconclusive findings from ileocolonoscopy and x-ray-based studies.

Aim: To explore the effectiveness of CE in the diagnosing of CD.

Methods: CE was performed for diagnostics CD in 15 patients, in whom there remained a high clinical suspicion of CD despite negative or inconclusive findings from ileocolonoscopy and/or radiological examinations. Findings compatible with CD by using CE included erosions, aphthoid or deep ulcers (more than 3) and strictures/stenosis.

Results: The procedure was performed in all patients. There weren't any complications and capsule retention. In 11 out of 15 patients revealed inflammatory changes of the small bowel, which were characteristic for the CD – aphthoid or deep ulcers, erythema, edema. These inflammatory changes were detected in the distal ileum. The increasing of the number of ulcers progressively in the distal ileum was characteristic.

Discussion/Conclusion: CE – an effective method in the diagnosing of CD.
Ferric carboxymaltose administration in iron deficient and anemic pediatric patients with IBD

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Introduction: Inflammatory bowel disease (IBD) in children tends to be more severe and extensive than in adults. Iron-deficiency anemia (IDA) is very common in pediatric IBD but is often tolerated and not adequately treated. This retrospective observational study is the first to describe the use of intravenous ferric carboxymaltose (FCM, Ferinject®) in pediatric IBD.

Methods: All subjects who had received treatment with at least 1 dose of FCM between 1 September 2008 and 31 December 2010 were included in this study with anonymized data collected for up to 3 month post last FCM dose.

Results: In total, 35 children (among them 19 with Crohn's disease and 11 with ulcerative colitis) between 2 and 18 years had been treated for IDA with a total of 77 doses of FCM. The mean cumulative dose was 911 mg iron (median single dose: 500 mg iron; max: 1000 mg) with single administration of up to 26 mg iron per kg body weight. Post administration haemoglobin (Hb) levels improved from 9.5 to 11.4 g/dl within 5–12 weeks. Decreases in white cell and platelet count and CRP post FCM administration potentially suggest reduced inflammation with iron repletion. Soluble transferrin receptor and reticulocyte Hb proved to be robust indicators for IDA and therapy response. Two subjects reported mild adverse events related to FCM, these were considered potentially related to long duration of administration and high volume of saline for dilution.

Conclusion: Overall FCM was effective in correcting iron deficiency anemia and was well tolerated also in pediatric IBD patients.
The phospholipids sphingomyelin and phosphatidylcholine contrarily affect the integrity of tight and adherens junctions in the murine intestinal mucosa during experimental inflammatory bowel disease

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Background: We recently reported that the phospholipids sphingomyelin and phosphatidylcholine, both being food components in a balanced Western diet, contrarily affect the induction of apoptosis in intestinal epithelial cells (IECs). Whilst sphingomyelin induces cathepsin D- and BID-mediated cell death and aggravates colitis in two mouse models of inflammatory bowel disease (IBD), phosphatidylcholine prevents IECs from dying. The relevance of epithelial damage for the mucosal barrier function and for the prevention of bacterial invasion and inflammation has been shown in numerous studies. We characterized the impact of sphingomyelin and phosphatidylcholine onto the function of the intestinal barrier with regard to intercellular junctions.

Methods: Acute dextran sulfate sodium (DSS) colitis was induced in C57-BL/6J mice over seven days. The animals daily received 4 mg sphingomyelin by oral gavage. IL-10⁻/⁻ mice that spontaneously develop a chronic colitis daily received 4 mg sphingomyelin or phosphatidylcholine for 21 days between postnatal week 11 and 14. BCL-2, caspase-3, and tight and adherens junction proteins were analyzed by Western blot and immunofluorescence.

Results: During DSS colitis and in IL-10⁻/⁻ mice sphingomyelin, but not phosphatidylcholine, significantly decreased the protein levels of anti-apoptotic BCL-2 in colonic tissue (-62.7 ± 15.2% and -56.2 ± 24.0%, respectively; both: p < 0.05). For IL-10⁻/⁻ animals, caspase-3 was significantly activated by sphingomyelin but remained unaffected by phosphatidylcholine treatment (+63.7 ± 14.3%, p < 0.05). This is in good accordance to our findings demonstrating the up-regulation of pro-apoptotic BID in response to orally applied sphingomyelin. Besides those apoptosis-related results, phosphatidylcholine strengthened the adherens and tight junctional complexes, as occludin, β-catenin and E-cadherin protein levels were significantly increased in colonic tissue of IL-10⁻/⁻ animals (+63.5 ± 12.4%, +183.9 ± 29.2%, and +31.1 ± 15.0%, respectively; all: p < 0.05). Whilst being present along the whole crypt in IL-10⁻/⁻ control and phosphatidylcholine-receiving mice, immunofluorescence revealed a loss of E-cadherin at the top of the crypts when sphingomyelin was applied.

Conclusion: The sphingomyelin-triggered aggravation of colitis in two animal models of IBD does not only result from the increased cathepsin D- and BID-mediated apoptosis, but also from weakened intercellular junctions and, thus, an impaired intestinal barrier. As the phospholipid phosphatidylcholine, being structurally related
to sphingomyelin, strengthened the junctional complexes and alleviated experimental IBD, we state that lipids may weaken or fortify the intestinal barrier, induce or prevent apoptosis, and positively or negatively influence colonic inflammation. Regarding food-derived phospholipids like sphingomyelin and phosphatidylcholine, personal nutritional habits may therefore influence the course of IBD.
Knock-out of BCL-2 interacting mediator of cell death (Bim) aggravates chronic DSS-induced colitis

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Background: Increased apoptosis of intestinal epithelial cells (IECs) and an extended lifespan of lymphocytes are considered to perpetuate inflammatory bowel diseases (IBD). In healthy cells pro-apoptotic BCL-2 interacting mediator of cell death (BIM) is bound to microtubule. Upon stress stimuli BIM is translocated to the mitochondrial surface to inactivate anti-apoptotic B-cell lymphoma-2 (BCL-2) and to initiate apoptosis. We investigated the impact of a Bim knock-out in the mouse models of acute and chronic dextran sulfate sodium- (DSS-) induced colitis.

Methods: Acute or chronic colitis was induced in female B6.129S1-Bcl2l11tm1.1Ast (Bim−/−) mice with 2% DSS for seven days or four seven-day cycles of 2% DSS interrupted by 14-day periods of recovery. Body weight loss, spleen weight, and colon length were recorded. Colonoscopy was performed and evaluated by the murine endoscopic index of colitis severity (MEICS). A histological score was determined. IECs and mesenterial lymph nodes (MLNs) were isolated and analyzed for apoptosis and cytokine profiling.

Results: For acute colitis, DSS-induced weight loss of Bim−/− mice was significantly decreased compared to wild type (WT) animals (p < 0.05) on day eight. In Bim−/− mice, shortening of the colon was clearly less pronounced and MEICS and histological scores were significantly lower as compared to WT controls (p < 0.05). Significantly less IECs of Bim−/− mice underwent apoptosis as compared to WT animals (p < 0.05).

For chronic colitis, MEICS and histological scores indicated an aggravated inflammation in Bim−/− animals as compared to WT controls, with significantly more lymphocytes infiltrating the colonic mucosa and submucosa (p < 0.05). Cytokine profiling in MLNs revealed that iNOS gene and protein expression in Bim−/− mice was significantly reduced as compared to controls.

Conclusion: As compared to WT animals, Bim−/− mice were protected from an acute experimental colitis, but had a worsened disease course when chronic inflammation was induced. As in Bim−/− mice apoptosis of IECs is reduced during acute colitis, we suggest a protection from a barrier defect in this situation. However, in chronic colitis, lymphocytes with a higher survival rate in Bim−/− mice may have aggravated and perpetuated the intestinal inflammation, pointing out the Janus-faced role of BIM in colitis and its possible potential as new treatment target in IBD.
Pregnancy and newborn outcome of mothers with inflammatory bowel disease exposed to anti-TNFα therapy during pregnancy: Three center study

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Introduction: Our aim was to assess the safety of anti-TNFα treatment on pregnancy and newborn outcome in women with inflammatory bowel disease (IBD) exposed to biologicals during pregnancy.

Methods: Women with IBD from three centres in Czech Republic exposed to anti-TNFs during pregnancy between January 2007 and October 2011 were included. Data on anti-TNFα treatment, disease activity, concomitant medication, pregnancy and newborn outcome were retrieved from medical files or prospectively recorded using predefined data collection form.

Results: 33 pregnancies were observed in 31 women (23 Crohn’s disease and 8 ulcerative colitis; median age 28 years), of which 25 (76%) were exposed to infliximab and 8 (24%) to adalimumab. At conception 9 (28%) women had active disease, 24 (72%) were in remission and 2 experienced relapse during pregnancy. Biologicals were initiated prior to conception in 26 women (median 41 weeks, range 2–251), while 7 patients started the therapy after conception (median 4 weeks; range 1–23). Out of all pregnancies, 28 were already terminated and 5 are still ongoing. Of the completed ones 24 (86%) ended in live births (median birth weight 3150 g; range 2400–4450) with 20 (71%) at-term and 4 (14%) pre-term deliveries. Three pregnancies ended in spontaneous abortion and one was terminated in 11th week (patient’s wish). No congenital malformations and no perinatal complication except for one neonatal jaundice were observed. The median time between last anti-TNFα application and delivery was 12 weeks (3–23). In 4 children infliximab levels from cord blood were assessed ranging from 1.8 to 25 mcg/ml. Three maternal complications were observed: 1 urinary tract infection requiring hospitalization, 1 gestational diabetes mellitus, and 1 newly diagnosed breast cancer.

Discussion/Conclusion: Pregnancy outcome was favourable and comparable to previous studies on anti-TNFs during pregnancy. Our results propose anti-TNFα therapy to be safe for pregnant women with IBD.
Climate change and the impact of heat waves on infectious gastroenteritis and relapse rates of inflammatory bowel disease

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Introduction: The health impact of heat waves (HW) has repeatedly been reported. Information on young patients’ illnesses as inflammatory bowel disease (IBD) is scarce. We therefore conducted this retrospective study to evaluate the effect of climate change on IBD flares. Because of reports on enteric pathogens’ influence on IBD we also investigated the incidence of infectious gastroenteritis (IG).

Methods: For the years 2001–2005 we collected data from 738 IBD, 786 IG and 506 non-infectious intestinally inflamed patients admitted to our hospital. Climate data was obtained from the Swiss Federal Office for Meteorology and Climatology. We used Poisson regression and time series analysis to evaluate the impact of HW on the incidence of IBD flares and IG, adjusting for day-of-the-week, long term trend and seasonal effects.

Results: Admittance due to flares of IBD showed a yearly increase of 14.2%. There was no evidence for a long-term time trend and only weak evidence for a yearly seasonal pattern peaking in winter (p = 0.059) for IG incidence. Presence of a HW increased the risk of IBD flares by 4.6% and by 4.7% for patients with IG for every additional day within a HW. In the control group there was no significant effect.

Discussion/Conclusion: In this first study on the impact of climate change on IBD and IG we found a substantial association between HWs and the risk to suffer from flares of IBD or IG.
CAD, a nucleotide synthesis enzyme, inhibits NOD2 anti-bacterial function in intestinal epithelial cells

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Introduction: Polymorphisms that impair the function of nucleotide-binding oligomerization domain (NOD)2, a bacterial sensor, are associated with Crohn's disease (CD). Proteins that selectively regulate NOD2 activity have yet to be identified. Using immunoprecipitation-coupled mass spectrometry to detect potential NOD2 regulators, we identified several interacting proteins, including carbamoyl phosphate synthetase/aspartate transcarbamylase/dihydroorotase (CAD).

Methods: CAD expression was assessed by immunohistochemistry of colon tissues from individuals with and without inflammatory bowel disease. Reporter gene assays and phospho-specific immunoblots measured NOD2-dependent NFκB and p38 MAPK activation in HCT116 intestinal epithelial cells. NOD2-dependent Salmonella killing was assessed in gentamicin protection assays. CAD and NOD2 expression were modulated by RNAi and expression plasmids. N-phosphonacetyl-L-aspartate (PALA) inhibited CAD enzyme activity. NOD2 CD-associated variants were expressed in HEK293T cells for analysis.

Results: CAD was identified as a novel NOD2-interacting protein. CAD expression was increased in the intestinal epithelium of CD patients as compared with controls (CD: 4.7 x 10^6 ± 2.9 x 10^6 vs. non-IBD: 2.1 x 10^5 ± 2.4 x 10^5 vs. UC: 1.4 x 10^6 ± 2 x 10^6 staining intensity/cell, p = 0.01). Increasing CAD expression inhibited NOD2-dependent activation of NFκB and p38 MAPK (NFκB: 5 x 10^5 ± 1 x 10^5 to 0.5 x 10^5 ± 6 x 10^3 light units, p = 0.002; p38: 1.2 x 10^4 ± 637 to 0.6 x 10^4 ± 386 light units, p = 0.0001). Reduction of CAD expression amplified phosphorylation p65 NFκB and p38 MAPK upon NOD2 stimulation. NOD2-dependent bacterial killing was also inhibited by CAD over-expression (8 x 10^4 ± 0.1 x 10^4 to 13 x 10^4 ± 1 x 10^4 cfu/well, p = 0.003), while killing was enhanced by RNAi knockdown of CAD (4 x 10^4 ± 0.3 x 10^4 to 1 x 10^4 ± 0.2 x 10^4 cfu/well, p = 0.001). Inhibition of CAD enzyme activity by PALA enhanced NOD2 anti-bacterial activity (5 x 10^4 ± 0.3 x 10^4 to 3 x 10^4 ± 0.2 x 10^4 cfu/well, p = 0.01). The impaired anti-bacterial function of CD-associated NOD2 variants was corrected by PALA (L1007fs: 6 x 10^4 ± 1 x 10^4 to 2 x 10^4 ± 0.5 x 10^4 cfu/well, p = 0.01).

Discussion/Conclusion: We identified CAD as a key regulator of host defense mediated by NOD2. Therefore, CAD could become a pharmacologic target to re-establish effective anti-bacterial function of CD-associated NOD2 variants and may offer a novel therapeutic strategy for CD.
Maltodextrin (MDX), a ubiquitous dietary additive in Western diets, enhances biofilm formation and adhesiveness of *E. coli*: A case for the environment altering the “in-vironment”

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**Introduction**: Crohn’s disease (CD) is a chronic inflammatory bowel disease with bacterial and environmental factors (such as diet) contributing to disease pathogenesis. A ubiquitous additive of Western diets is MDX, a polysaccharide derived from starch hydrolysis. One striking difference between the healthy gut and one affected by CD is the formation of dense bacterial biofilm structures in intimate proximity to the gut epithelium. We investigated whether MDX enhances biofilm formation and cellular adhesion of CD-associated *E. coli* strains.

**Methods**: Laboratory and clinical *E. coli* isolates, including the CD-associated LF82 strain, were grown in M9 minimal media supplemented with glucose or MDX for 24 h. Specific biofilm formation was assessed in microtiter plate assays. Adhesion of LF82 to HT29 or Caco2 monolayers was measured 1-6 h post-infection (MOI = 10). *E. coli* were visualized by scanning electron microscopy. Type I pili expression was evaluated by FimA/E invertible element PCR and function evaluated by mannose blockade and mutant strains. CEACAM6 expression was knocked down by RNAi.

**Results**: MDX dramatically enhanced specific biofilm formation of LF82 *E. coli* (0.2 ± 0.2 to 1.4 ± 0.1, p = 0.0003). This effect was not unique to LF82, as MDX promoted biofilm formation of other *E. coli* strains. MDX enhanced bacterial adhesion to epithelial monolayers as early as 3 h, peaking at 6 h (6 x 10^6 ± 4 x 10^6 to 23 x 10^6 ± 5 x 10^6, p = 0.01). MDX induced thin, hair-like projections from the bacterial surface similar to type I pili. MDX stimulation of type I pili expression was confirmed and functional blockade abrogated both MDX-enhanced biofilm formation and cellular adhesion. Surprisingly, RNAi knockdown of the previously characterized cellular receptor, CEACAM6, failed to alter MDX-enhanced adhesion, indicative of a unique mechanism of adherence.

**Discussion/Conclusion**: Our findings demonstrate that a ubiquitous component of Western diets strongly promotes pathogenic phenotypes in CD-associated bacterial strains, suggesting a mechanism by which environment changes the bacterial “in-vironment” to promote disease.
Cognitive-behavioral therapy changes the “in-vironment”

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Introduction: Recent animal studies provide evidence for the relationship between stress, depression and gastrointestinal inflammation. Therefore, improving psychological status in IBD may not only improve quality of life but also delay or prevent relapse of IBD and thus be associated with improved physical outcomes and decreased inflammatory activity. This paper presents the preliminary data on the efficacy of a cognitive-behavioral therapy (CBT) program designed to improve both psychological and disease status in IBD patients.

Methods: The study is a randomized controlled trial. Adult patients with quiescent/mild disease were randomly allocated to either CBT (experimental group) or usual medical care (controls). The experimental group had 10 weekly CBT sessions either face-to-face or online. Both groups were reassessed at 6 and 12 months.

Results: To date, 45 controls and 37 experimental group participants have been enrolled in the trial and the study is still recruiting. A significant improvement in disease activity in the experimental group participants with CD but not in controls between baseline, 6 and 12 months was observed (p < 0.05). In this period of time, the following trends were also noted: anxiety, depression and maladaptive coping dropped and mental quality of life improved in the experimental group with no such change in controls.

Discussion/Conclusion: CBT program designed specifically for IBD patients seems to be an effective tool in reducing disease activity and has a potential to improve a broad spectrum of psychological parameters. However, a bigger sample needs to be reached before final recommendations are made.
IRF4\textsuperscript{-/-} mice develop higher tumor number and score in the colitis-associated tumor model than wildtype control mice

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Introduction: In previous studies we have shown that IRF4\textsuperscript{-/-} mice produce reduced amounts of IL-6 in two models of chronic inflammation (oxazolone/-TNBS-induced colitis) (Mudter et al., JCI, 2008). In contrast no differences in IL-6 levels were found between IRF4\textsuperscript{-/-} and wildtype (WT) mice after dextran sodium sulfate (DSS) treatment (Mudter et al., Inflamm Bowel Dis, 2010). In this study we analyze the role of IRF4 in tumor development in the AOM/DSS tumor model. Becker et al. (Cell Cycle, 2005) have shown that TGF-\(\beta\) production in tumor infiltrating T lymphocytes suppresses tumor growth in the colon via inhibition of IL-6 production by T cells.

Methods: Eight- to twelve-week-old sex-matched IRF4\textsuperscript{-/-} and C57BL/6 WT mice were used. To induce colon carcinomas mice received a single intraperitoneal injection of the mutagenic agent azoxymethane (AOM) (3 mg/kg body weight), three cycles of 2% DSS in drinking water for one week and normal drinking water for two weeks. For the monitoring of tumorigenesis a video miniendoscope was used. Scoring of tumor development was based on the tumor number and size. Quantitative PCR of IL-6 was performed.

Results: Treatment of 2% DSS in drinking water induced weight lose in IRF4\textsuperscript{-/-} and WT mice. The tumor number and score, for the latter a value is assigned according to the number of tumors, was significantly increased in IRF4\textsuperscript{-/-} mice compared to WT mice. The tumor size score which takes the number and size of tumors per animal into account was not different. No significant differences in IL-6 mRNA levels were detected between AOM/DSS-treated WT and IRF4\textsuperscript{-/-} mice.

Discussion/Conclusion: IL-6 cannot be the reason for increased tumor number in IRF4\textsuperscript{-/-} compared to WT mice since no significant differences in IL-6 levels were detected. Hence another mechanism must be the reason for the increased tumor number in those mice.
The comparison expression of proapoptotic proteins (Bak and Bax) and anti-apoptotic Bcl-XL protein in inflammatory bowel diseases

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Introduction: Literature describes significant role of apoptosis in the evolution of pathogenesis of ulcerative colitis and Crohn’s disease. Apoptosis or programmed cell death is the major genetically regulated process of cell self-destruction. Apoptosis is regulated by caspase activation and it depends on the action of other proteins of the Bcl-2 family, including both caspase-activating enzymes (Bax, Bak) and caspase-inhibiting proteins (Bcl-xL). The study objective was the immunohistochemical assessment of the expressions of the apoptosis-regulating proteins Bak and Bax as well as anti-apoptotic Bcl-XL protein in inflammatory bowel diseases.

Materials and methods: The study group consisted of 18 patients with ulcerative colitis (UC) and 10 subjects diagnosed with Crohn’s diseases (CD). The levels of Bak, Bax and Bcl-XL expression were determined immunohistochemically. The expressions of these proteins were observed in cytoplasm of cells.

Results: The increased in expression of Bax and Bak proteins in dysplastic cells of patients with Crohn’s diseases and ulcerative colitis compared to the normal epithelium was observed. The higher expression of anti-apoptotic Bcl-XL protein in dysplastic cells in both diseases was found too. What is more, the Bcl-XL expression was stronger in both normal and dysplastic cells opposite to the expression of proapoptotic proteins in these diseases.

Conclusions: On the basis of our observations, we may suggest that changes in the expression of the proapoptotic proteins (Bak and Bax) and anti-apoptotic Bcl-XL protein can take part in development of dysplastic cells in Crohn’s diseases and ulcerative colitis.
Further studies on the serum neutrophil gelatinase-associated lipocalin-2 (NGAL) in children with inflammatory bowel disease (IBD)

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NGAL, anti-bacterial and cytomodulatory protein, is expressed on various epithelial cells. Its secretion is rapidly upregulated in pathophysiology in response to cellular injury. Recently, we have shown for the first time (Falk Symposium No 179, 2011) that in IBD children the serum NGAL level is highly elevated.

Aim: To investigate whether serum NGAL concentration correlates with the common indices of IBD.

Methods: Diagnosis of IBD was established based on the Porto criteria. Blood samples were obtained from children with Crohn’s disease (CD, n = 19), and ulcerative colitis (UC, n = 18) for the diagnostic examinations. It was possible to use a part of each sample for this study. Serum NGAL level was quantified with ELISA kit (Bioponto, Denmark).

Results: Serum NGAL level was highly elevated in our IBD children: 116 ± 71.3 (range 37.3–405 ng/ml) compared to healthy (38.8 ± 12.5, n = 15) and non-IBD, allergic (tIgE: 300–5000 kIU/l) children (37.1 ± 12.5, n = 13). In CD (n = 19) the level was 103 ± 57.2 whereas in UC (n = 18) it was 130 ± 84.2 ng/ml (p > 0.05). In children with Pediatric CD Activity Index (PCDAI) 2.5–30, and > 30 the NGAL level was 83.7 ± 35.9 and 111 ± 61.9 ng/ml, (p > 0.05). In children with Pediatric UC Activity Index (PUCAI) 0–30, and > 30 the NGAL level was 135 ± 106 and 122 ± 33.7 ng/ml, respectively (p > 0.05). There was no significant correlation between serum NGAL and C-reactive protein, erythrocyte sedimentation rate, hemoglobin, platelets, leukocytes and disease activity indexes.

Discussion/Conclusion: Unlike Oikonomou et al., (J Gastroenterol, 2011) we did not find statistically significant correlations between elevated serum NGAL concentration in children with CD and UC and common indices of inflammation and IBD severity. A role of NGAL in pathophysiology of the disease requires further investigations.
Intestinal neuronal dysplasia as a rare cause of intestinal obstruction: Case report

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Introduction: A young man with intestinal obstruction diagnosed as intestinal neuronal dysplasia (IND) is presented here.

Case report: A 24-year-old man was admitted to our hospital with the complaints of poorly localized diffuse abdominal pain for 2 years, subsequent intermittent diarrhea, fatigue and weight loss of 8 kg. He had undergone appendectomy 2 years ago. He had been using oral coumadin 5 mg/day upon the diagnosis of deep venous trombosis for 6 months. On physical exam, he seemed well, with stable vital signs. He had no skin lesion apart from previous appendectomy scar. The abdomen was tender at the right lower quadrant and the bowel sounds was increased. Initial laboratory tests showed anemia (hemoglobin 12.9 g/dl), hypoalbuminemia (albumin 2.7 g/dl) and hypokalemia (potassium 3.3 mEq/L). Serum levels of other biochemistries, serologic parameters, hemostasis parameters, stool and urine analysis were all within normal limits. Further exams were planned with the assumption of Crohn's disease. Abdominal X-ray, ultrasound and CT revealed air-fluid levels, wall thickness of ileocecal segment and colocolonic invagination at the descending colon (Figure 1). Colonoscopy showed the narrowed segment at the level of probable invagination. The patient underwent surgery which revealed thickness of the cecal bowel wall and narrowing of the terminal ileum and a right hemicolectomy and ileocolonic anastomosis was performed. Pathology was reported as intestinal neuronal dysplasia upon the findings of hyperplasia in the neural tissue and ganglion cells (Figure 2), and NSE, synaptophysin and S100 positivity (Figure 3). He is doing well after operation.

Discussion: IND is a disease of the submucosal plexus of intestine manifesting chronic intestinal obstruction or severe chronic constipation, mainly in the children. It is one of intestinal dysganglionoses and clinically closely associated with Hirschsprung's disease. It is not clear whether it is a congenital malformation or an acquired secondary condition related to some gastrointestinal problems. Adult forms of IND is very rare, and if occurs its usual presentation is nonspecific like constipation. In the literature, only one case of IND with intestinal obstruction was reported like our patient.
Do localization of inflammatory bowel disease predict the hepatobiliary manifestation?

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Aim: To evaluate the predictive factors of hepatobiliary manifestation in inflammatory bowel disease (IBD) patients.

Methods: Between 1999–2009 years, patients diagnosed with IBD were analyzed retrospectively. Demographics, clinical and laboratory data were evaluated. Primary sclerosing cholangitis (PSC) were diagnosed by biochemical analyses, MRCP/ERCP and liver biopsy in all suspected patients. Hepatosteatosis was diagnosed by ultrasound.

Results: 504 patients (51.8% male) with IBD (57.5% with ulcerative colitis, 39.1% Crohn's disease and 3.4% indeterminate colitis) were enrolled in this study. Mean age of patients was 38.7 ± 13 years. Mean duration of disease and follow-up period were 80 ± 59 months and 32 ± 3 months, respectively. Hepatobiliary manifestations rate was 4.8% (24 pts) (66.7% ulcerative colitis, 33.3% Crohn’s disease). PSC was diagnosed in half of them and hepatosteatosis in the other patients. In this group 58.3% of them were male and mean duration of disease was 32 ± 3 months (2–96 months). All PSC patients received UDCA (15 mg/kg) median 84 months. Mean ALP (397 ± 507 IU/L vs. 271 ± 255 IU/L) and GGT (87 ± 92 IU/L vs. 68 ± 84 IU/L) levels were significantly decreased after UDCA treatment (p < 0.05). There was a positive correlation between duration of disease and hepatobiliary manifestation (r = 0.1, p = 0.025). There was no correlation between hepatobiliary manifestation and gender. Univariate analysis revealed ileocolonic involvement and pancolitis were independent risk factor for the development of hepatobiliary manifestations in Crohn’s disease and ulcerative colitis, respectively (p = 0.005). During the following period there was no hepatic failure.

Conclusion: Colonic involvement is a risk factor for hepatobiliary manifestation in inflammatory bowel disease.
Natural course in Crohn’s disease under the conventional therapy

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Aim: To investigate demographic and clinical characteristics of Crohn’s disease (CD) patients in a tertiary reference center.

Material and methods: Data from CD patients who presented to Istanbul Medical Faculty Gastroenterohepatology Inflammatory Bowel Disease (IBD) clinic between years 2006 and 2010 were retrospectively analyzed.

Findings: A total of 225 patients (58% male) were included in the trial. Mean age was 35.6 years (17–72), mean age-of-onset was 63 months (0–350) and mean follow up time was 27 months (6–72). One percent of patients had a family history of IBD. Sixty-three percent of patients presented with acute onset while 37% was referred from other centers. Based on Montreal classification, 60% had ileocolonic, 25% had ileal, 7% had colonic, 6% had perianal and 2% had upper gastrointestinal Crohn’s disease. Disease phenotypes were as follows: 58% inflammatory, 23.5% fistulising, 18.5% stenosing at presentation and 53% inflammatory, 34% fistulising, 13% stenosing at the end of follow-up period. Eighty-five percent of patients was treated with 5-ASA, 45% on steroids + azathiopurine, 13% on steroids and 22% biological agents. Only one and half percent of patients received biological agents as first line therapy.

Conclusion: A greater percentage of CD patients present with an acute onset at a young age and have ileocolonic disease localization. In inflammatory pattern dominant CD, progressed to fistulising phenotype even under treatment. It could be explained by the lack of biological agents in the therapy of this group. These data show that although immunosuppressive agents are partially useful, they do not have the power to change the natural course of disease.
A young patient with acute pancreatitis, B hepatitis, dyslipidemia and bloody stools

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Introduction: Pancreatitis is a potentially severe condition. The incidence of pancreatitis is increased in inflammatory bowel disease (IBD). However, pancreatitis as an extraintestinal manifestation of the intestinal disease is exceedingly rare. Acute pancreatitis occurring during the course of inflammatory bowel disease may be idiopathic, related to drugs used in the treatment of these patients, biliary lithiasis, primary sclerosing cholangitis and duodenal involvement from Crohn's disease. Asymptomatic elevation of serum amylase on patients with IBD has also been noticed. So far, acute pancreatitis has been described only after the establishment of diagnosis of IBD.

Methods: We describe a patient with IBD in whom acute idiopathic pancreatitis developed before the onset of symptoms of the underlying IBD.

Results: A 36-year-old man developed an episode of acute pancreatitis. Diagnosis was based on the relevant clinical picture and the increased serum lipase levels. Diagnosis was also supported by the relevant abdominal ultrasound and CT scan. Upper and lower GI endoscopy were normal. Other causes of pancreatitis were excluded with appropriate investigation. The treatment for pancreatitis relieved the pain and decreased the serum levels of pancreatic enzymes to within normal ranges. The patient was discharged with no symptoms and normal serum and urine amylase. After three months, he started complaining of diarrhea and anal bleeding. Colonoscopy and histopathologic examination revealed ulcerative colitis in the rectum and treatment with Salofalk® made proctitis quiescent. In a 18-month follow-up period, proctitis and pancreatitis didn’t relaps.

Discussion/Conclusion: In conclusion, we suggest that acute idiopathic pancreatitis may precede the clinical manifestations and diagnosis of the underlying IBD. Acute pancreatitis of idiopathic origin in young patients could be considered as an early extraintestinal manifestation of as-yet undiagnosed IBD.
The role of caspase-8 in inflammatory bowel diseases

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Introduction: Caspase-8 is one of the proteins that initiate apoptosis, a physiological process of the programmed cell death. Moreover, it participates in the lymphocytes activation. Its deficiency or disturbance in the functioning can result in the extension of the inflammatory process. In tumors, the lack of this enzyme contributes to the prolongation of cancer cells life and tumor development. Therefore, the purpose of the research was to evaluate the expression of caspase-8 in inflammatory bowel diseases that are threatened with the development of colorectal cancer.

Material and method: The study included a group of 10 patients diagnosed with Crohn's disease and 31 patients with ulcerative colitis. The expression of caspase-8 in tissue material was evaluated and determined by the immunohistochemical technique. The staining reaction was observed in details in the surface epithelium, normal and dysplastic glands and in inflammatory cells. The score of immunohistochemistry expression was 4-step: the reaction to caspase-8 was absent, weak, medium and strong.

Results: In patients with ulcerative colitis it was observed: the weak and the medium expression of caspase-8 in the surface epithelium (38.7% and 38.7%), the absence and the weak expression in normal glands (41.9% and 32.3%), predominant weak and medium reactions in dysplastic glands (33.3% and 50%), and weak in the inflammatory cells (58%). In turn, the patients diagnosed with Crohn's disease had a strong expression of caspase-8 located in the surface epithelium in 80% of cases. Moreover, in the normal glands the expression defined as weak, medium and strong (30%, 40% and 20%, respectively) was noted. Only one patient was found with a dysplastic glands where the strong expression of caspase-8 was observed. The strong reaction of inflammatory cells to caspase-8 was observed in up to 90% of patients with Crohn's. Statistical analysis showed a correlation between the increase in caspase-8 expression in normal glands with its growth in the surface epithelium (p = 0.035) and in inflammatory cells (p = 0.033).

Conclusion: A stronger expression of caspase-8 in Crohn's disease than in ulcerative colitis was the evidence of the increased induction of apoptosis in this disease process. Whereas, in our opinion, the increased expression of caspase-8 in inflammatory cells in Crohn's disease may be associated with the increase in the number of lymph nodules as a response to chronic inflammation.
The metabolic syndrome in patients with IBD as compared with patients with colorectal cancer

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Introduction: It is known that the hypercaloric and hyperlipidic diet, together with obesity, dyslipidaemia and diabetes mellitus are involved in the appearance of the colorectal cancer (CRC). Our aim was to establish if these risk factors are also involved in the pathogenesis of inflammatory bowel diseases (IBD), as very few studies on this matter have been made in Romania.

Methods: We have retrospectively analyzed all colonoscopies which were performed during the last 4 years in our Gastroenterological Department. Regarding the components of the metabolic syndrome, we have analyzed the glycemic level, the cholesterol and triglycerides levels in patients with IBD, as compared with patients with CRC.

Results: From the total number of 911 patients, 3.33% were diagnosed with CRC and 1.53% patients were diagnosed with IBD (21.42% of them with Crohn’s disease and 78.58% with ulcerative colitis). The medium age of the patients with IBD was 55.92 ± 13.35 years. The gender distribution was 57.14% women and 42.86% men. The haemoglobin level in patients with IBD was significantly lower than in patients with CRC (p = 0.017). The medium glycemic level was 99.08 mg/dl in patients with IBD, as compared with 117.25 mg/dl in patients with CRC (p = 0.032). The medium cholesterol level was significantly lower in patients with IBD, as compared with patients with CRC (149.83 mg/dl as compared with 190.17 mg/dl; p = 0.012). The medium triglycerides level was significantly lower in patients with IBD, as compared with patients with CRC (70.4 mg/dl, as compared with 157.05 mg/dl; p = 0.016).

Discussion/Conclusion: In our area, inflammatory bowel diseases have a low incidence; ulcerative colitis is three times more frequently than Crohn’s disease. The metabolic syndrome components are less involved in the IBD pathogenesis than in CRC.
Elevated levels of serum angiotensin converting enzyme (ACE) in patients with ulcerative colitis (UC) as a clue for activated renin-angiotensin system (RAS) in inflammatory bowel diseases

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Introduction: Ulcerative colitis (UC) is a systemic disease comprising inflammation of the gastrointestinal (GI) tract of unknown etiology. The renin-angiotensin system (RAS) is stringently associated with the kallikrein-kinin system and are convoluted in many physiological and disease conditions (Figure 1) and possibly in the pathogenesis of inflammatory bowel disease (IBD). Recent data indicates that RAS is well conveyed and active in the GI tract although exact physiological functions are to be established. Of particular interest is the increasing amount of experimental support for the involvement of Ang II formation and actions via the Ang II subtype 1 (AT1) receptor in the pathogenesis and treatment of IBD. Angiotensin-converting enzyme (ACE), an important molecule of RAS, is also known as a regulatory molecule in distinct disorders. The aim of the present study was to investigate the possible role of the ACE in the context of RAS in UC pathogenesis.

Methods: The study group comprised 47 UC patients, with 20 controls. Median age of UC and healthy controls were 53.5 (25–68) and 55 (24–72) respectively. ACE was measured by monitoring the alteration in absorbance at 340 nm of the hydrolysis of furylacryloylphenylalanlylglycylglycine (FAPGG) to FAP and GG on an analyzer. The ACE activity in the sample was determined by comparing the sample reaction rate to that obtained with the ACE calibrator. Complete blood count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were determined for both patients and controls.

Results: Serum mean ACE levels were 70.6 ± 13.6 and 51.8 ± 26.7 U/l for patients with UC in active state and in remission and 29.9 ± 9.3 U/l in the healthy control group respectively (Figure 2). Mean serum ACE levels were significantly elevated in active UC patients compared with patients with UC in remission and control groups.

Discussion/Conclusion: Serum ACE levels were found to elevate in UC patients in active state suggesting a partial role of RAS system in the disease pathophysiology. ACE can be used as a supportive diagnostic marker in patients with UC. Further randomized controlled studies are warranted to demonstrate the role of ACE in UC patients with a special interest in specific targeted therapies against ACE for achieving disease remission.
**Figure 1**: Potential contribution of renin-angiotensin system (RAS) to the physiological and disease conditions.

**Figure 2**: Serum ACE levels in patients with UC with active state and in remission with control group.
A retrospective study comparing the prevalence and characteristics of the inflammatory bowel diseases and colorectal cancer at different age groups of patients from southern Transylvania

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Introduction: The aim of this study was to establish the prevalence and characteristics of the patients with inflammatory bowel diseases (IBD) and colorectal cancer (CRC) in our geographical area, as few studies have been performed on this topic in southern Transylvania.

Methods: We have retrospectively analyzed all colonoscopies which were performed during the last 4 years in our Gastroenterological Department. We have analysed the demographic characteristics of the in patients with IBD, as compared with patients with CRC.

Results: From the total number of 911 patients, 3.33% were diagnosed with CRC and 1.53% patients were diagnosed with IBD (21.42% of them with Crohn's disease and 78.58% with ulcerative colitis). The medium age of the patients with IBD was 55.92 ± 13.35 years. The gender distribution of the patients with IBD was 57.14% women and 42.86% men. 42.86% were from rural areas, while 57.14% live in urban areas. 64.28% of the patients received maintenance therapy with mesalazine (1.5 g/day orally or suppositories). The studied lot was divided into two groups: 318 patients over 70 years, 593 patients which were less than 70 years of age. The relative risk of developing a colorectal cancer at the patients older than 70, compared with those less than 70 was 1.73. The relative risk of finding an inflammatory bowel disease at older patients compared with younger ones was 0.5, so this group had a lower risk of IBD.

Discussion/Conclusion: Inflammatory bowel diseases have a low incidence in Romania; ulcerative colitis is three times more frequently than Crohn's disease. IBD have a higher incidence in women and in urban areas. The inflammatory bowel disease is two times more likely to appear in the younger patients than in the elder ones. In contrast, the colorectal cancer is almost two times more frequently in elderly patients.
Molecular effects of the prebiotic «Zacofalk NMX®» in patients with ulcerative colitis

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Introduction: Nowadays the incidence of ulcerative colitis (UC) in Russia is 5–30 cases per 100,000 per year. The purpose of the work was the research of molecular effects of «Zacofalk NMX®» (calcium salt of butyric acid – 250 mg, inulin – 250 mg) concerning the enterome and the blood proteome in patients with UC.

Methods: The clinical trial was spent according to GCP. It has been included 63 patients with UC in the phase of the remission. Patients accepted mesalazine (Salofalk®) 1.5 g daily and «Zacofalk NMX®» 1.36 g 3 times a day, 40 minutes before meals. Criteria of the diagnosis of UC corresponded to diagnostic optimum. Additional researches are executed: fluorescent hybridization in situ, capillary gas-liquid chromatography, MALDI-TOF-TOF-MS. Control group – 20 healthy persons. The duration of the trial is 30 days. Statistical processing was spent on the basis of the program "Statistica 7.0".

Results: The reduction of Escherichia coli, Proteus spp., Enterococcus spp., Staphylococcus spp., Streptococcus spp., Bacteroides spp., Clostridium spp. and the normalization of Bifidobacterium spp., Lactobacterium spp. are registered in fecal samples of patients with UC for 30 day. Statistically significant reduction of the n-butyrate concentration and the increase of propionate concentration in fecal samples of patients are noted for 30 day. The increase of the expression of PPARγ, MUC2, MCT1, CFTR, HDAC and the decrease of GLUT 1, NF-κB, NRP-1, IL-1b, TNF-α, IL-2, IL-6, IL-8, IL-12 in blood serum is revealed for 30 day.

Discussion/Conclusion: Additional reception of «Zacofalk NMX®» in standard mode of the UC treatment is eliminated of dysbiotic relationship of protective and aggressive bacteria, it has shown metabolic and anti-inflammatory effects on extraintestinal level.
Plasma thrombin activatable fibrinolysis inhibitor (TAFI) could be a potential indicator of disease activity in ulcerative colitis

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Introduction: Ulcerative colitis (UC) is a chronic inflammatory disease primarily affecting the colonic mucosa and characterized by recurrent inflammation and remissions and relapse, which may occur as a result of medical treatment or spontaneously. Many non-invasive tests have used for monitoring the disease activity and inflammation.

In addition to suppressing fibrinolysis, thrombin activator fibrinolysis inhibitor (TAFI) was suggested to be involved in inflammation. To date, a few studies have been published that reports the role of TAFI in ulcerative colitis. Therefore, the objective of the present study was to investigate the role of plasma TAFI as an indicator of inflammation in UC, and its association with disease activity.

Methods: Twenty patients with UC and 17 healthy controls attending at the Gastroenterology Clinic of Turkiye Yuksek İhtisas Teaching and Research Hospital, Ankara, Turkey, between May 2010 and April 2011 were enrolled in the study. Plasma TAFI antigen levels were quantitatively determined by using ELISA kits in 20 UC patients at activation and remission and 17 healthy controls. Associations of TAFI with inflammatory markers to determine UC activation was assessed. To predict and determine the activation of UC, endoscopic activation index (EAI) and Truelove-Witts index were used for each subject.

Results: Plasma TAFI levels was higher in UC patients at activation of the disease compared with healthy controls. The disease activity according to EAI and Truelove-Witts index were significantly correlated with TAFI levels (rs: 0.721, p < 0.001 and rs: 0.537, p < 0.001 respectively). Overall accuracy of TAFI in determining UC activation was 85% with a sensitivity, specificity, NPV and PPV of 85%, 85%, 85%, and 85% respectively (cut-off value: 156.2 and AUC: 0.882).

Discussion/Conclusion: The present study demonstrated that TAFI is elevated in activation of UC. The appraisal of TAFI levels in patients with UC in conjunction with other markers of inflammation may provide additional information in estimating UC activation and severity.
Activation of protein tyrosine phosphatase non-receptor type 2 by the polyamine, spermidine, ameliorates IFNgamma-induced proinflammatory effects in human THP-1 monocytes

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Background: The gene locus encoding protein tyrosine phosphatase non-receptor type 2 (PTPN2) has been associated with inflammatory bowel disease (IBD). We have previously shown that PTPN2 is activated by interferon gamma (IFNγ) and regulates IFNγ-induced signalling and cytokine secretion in human intestinal epithelial cells and monocytes. Overactivation of immature immune cells has been demonstrated in CD and elevated levels of proinflammatory cytokines, such as interleukin-6 (IL-6) and macrophage chemoattractant protein 1 (MCP-1) play an important role in the pathophysiology of IBD. Polyamines, such as spermidine, have been demonstrated to activate PTPN2. The aim of this study was to investigate whether activation of PTPN2 by spermidine could be able to ameliorate IFNγ-induced proinflammatory signalling and cytokine production in human THP-1 monocytes.

Methods: Protein analysis was performed by Western blotting, mRNA analysis by real-time PCR. PTPN2 knock-down was induced by siRNA and cytokine levels were measured by ELISA.

Results: IFNγ (1000 U/ml) treatment of THP-1 cells induced phosphorylation (activation) of signal transducers and activators of transcription (STAT) 1 (p < 0.001, n = 3) and 3 (p < 0.001, n = 3) as well as of the mitogen-activated protein kinase isoform, p38 (p < 0.05, n = 3), by treatment for 30 min. Co-administration of the polyamine, spermidine (10 µmol) diminished the IFNγ-induced phosphorylation of STAT1 (p < 0.001, n = 3), STAT3 (p < 0.05, n = 3) and p38 (p < 0.05, n = 3). On a functional level, IFNγ treatment for 24 h resulted in increased mRNA levels of intercellular adhesion molecule 1 (ICAM-1, p < 0.001, n = 3) and interleukin-6 (IL-6, p < 0.01, n = 3). Co-treatment with spermidine prevented the cytokine-induced rise in ICAM-1 (p < 0.05, n = 3) and IL-6 (p < 0.05 vs. IFNγ alone) and monocyte chemoattractant protein 1 (MCP-1, p < 0.01 vs. IFNγ alone) after 24 h. Of note, in PTPN2-deficient cells, co-administration of spermidine had no effect on IFNγ-induced STAT1 phosphorylation and secretion of MCP-1.

Conclusions: In summary, our data demonstrate that PTPN2 activation by the polyamine, spermidine, effectively ameliorates IFNγ-induced STAT/MAPK-signalling and cytokine secretion. These findings support the hypothesis that activation of PTPN2 exerts anti-inflammatory effects in human monocytes. Our data suggest that activation of PTPN2 could provide a therapeutic approach for the treatment of chronic inflammatory disorders, such as IBD.

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The role of TNF-α and IL-2 in ulcerative colitis

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Introduction: Cytokines mediate tissue injury in various forms of colitis and play a key role in the pathogenesis of ulcerative colitis (UC) and are the most revealing of the immune defects found in inflammatory bowel disease (IBD). In this study we tried to clarify the role of tumor necrosis factor alpha (TNF-α) and interleukin-2 (IL-2) in ulcerative colitis in comparison to non-specific colitis (NSC).

Methods: The study was conducted on 56 patients. Group I included 30 patients with active UC. Group II included 16 patients with all other forms of colitis and called the non-UC group. Ten patients with symptoms related to irritable bowel syndrome (IBS) and with normal colonoscopy served as control (group III). Cases underwent clinical, laboratory, endoscopic and histopathological assessment of colonic biopsies including immunohistochemical staining for TNF-α and IL-2.

Results: A higher expression of TNF-α in the UC group (group I) (100%) as compared to the non-UC group (group II) (62.5%) (p = 0.01) was found. No significant difference of expression of IL-2 was detected between both groups (26% and 31%, respectively). On the other hand there was no expression of either cytokines in the control group (group III). Severity of TNF-α expression correlated with symptoms and histopathological changes of UC.

Discussion/Conclusion: TNF-α immunoreactivity is a significant feature of UC patients. The significant correlation of TNF-α with some clinical and histopathological criteria of UC builds an important theory for the pathogenesis of UC and targets future therapy options of the disease.
Expression of TNFR1 in colonic epithelium in inflamed mucosa of patients with Crohn’s disease

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Introduction: Inflammatory bowel diseases are characterized by enhanced epithelial loss, what leads to the impairment in barrier function of the gut mucosa. Probably this phenomenon is partly a consequence of increased apoptosis of epithelial cells. The aim of the study was to assess the intensity of epithelial apoptosis in inflamed mucosa in patients with Crohn’s disease (CD) and its relation to expression of TNFR1 (tumor necrosis factor alpha receptor 1) in colonocytes.

Methods: 35 patients with CD were enrolled in the study. Colonoscopy was performed in each patient and tissue sampling from inflamed colonic mucosa was made for histological analysis. Control group consisted of healthy people – samples of normal colonic mucosa were obtained during screening colonoscopy. Expression of active caspase 3, as a molecular marker of apoptosis, and expression of TNFR1 were assessed using immunohistochemical methods. Data were analyzed statistically.

Results: In colonic biopsies taken from inflamed mucosa of patients with CD epithelial expression of active caspase 3 and TNFR1 was significantly higher than in the control group, however there was no correlation between expression of these proteins.

Discussion/Conclusion: In CD there is an increase of colonic epithelial apoptosis in inflamed areas of the mucosa and probably this is one of the main mechanisms leading to mucosal barrier dysfunction in CD. The overexpression of TNFR1 in this group suggests, that enhanced colonocytes apoptosis is at least partly mediated via extrinsic TNFR-1-mediated apoptotic pathway.
The potential role of nitric oxide system in inflammatory bowel disease associated colorectal carcinogenesis: A meta-analysis

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Introduction: Inflammatory bowel diseases (IBD) including long-standing ulcerative colitis (UC) have an elevated risk of evolving into colon cancers. The reactive oxygen species, in particular nitric oxide (NO) system, generated by inflammatory cells create oxidative stress and contribute to neoplastic transformation, proliferation, and even metastasis. Apart from being a major mediator of chronic inflammation, NO modulates tumorigenesis and regulates cell proliferation, angiogenesis, survival, migration, and DNA repair. Regardless from the etiology of the disease, oxidative and nitrative DNA damage takes place at the sites of tumorigenesis. Furthermore inducible NOS mediated excessive amounts of reactive oxygen species production during chronic inflammatory conditions may play a crucial role in tumorigenesis by also causing DNA injury. In the present meta-analysis, we aimed to analyze the relationship between NO and IBD associated carcinogenesis and the effect of NO inhibition on disease management.

Methods: A systematic search of electronic databases up to January 2012 was performed to identify all primary studies examining the role of NO system in IBD. All articles were critically appraised with regard to methodological quality and risk of bias. Fifteen clinical trials that fulfilled the inclusion criteria were further pooled into a meta-analysis.

Results: Seventeen studies met initial selection criteria but only 9 were eligible for inclusion in the meta-analysis. The majority of studies demonstrated a significant role of NO (either iNOS or eNOS) in the pathophysiology of IBD associated carcinogenesis. Table 1 summarizes the components of the NO system their selected representative interrelationships with IBD associated tumoral development and the effect of iNOS blockade on disease management.

Discussion/Conclusion: In the present meta-analysis evidence suggests that activation of inflammatory cells which leads to release of proinflammatory and anti-inflammatory mediators are the probable source of NO production in IBD patients. Furthermore, elevated NO levels in IBD associated malignant patients suggests that NO due to iNOS/eNOS activation can be carcinogenic. This effect may be from NO associated stimulatory effects on angiogenesis, cell proliferation and invasion as well as inhibitory effects of NO on apoptosis. Further studies that will specifically focus on the role of NO system in the development of IBD associated carcinogenesis are strongly needed which may be a novel therapeutic target for the treatment of IBD.
Figure 1: Roles of nitric oxide (NO) in carcinogenesis. eNOS can be stimulated by post-translational modification (e.g., ERK 1/2 or PIK/AKT pathways) or by transcription (e.g., NF-κB). Based on the cellular milieu, release of low concentrations of NO can directly damage DNA, inhibit DNA repair, block apoptosis, stimulate angiogenesis and enhance oncogene expression. Moreover eNOS may also play a substantial role in tumoral metastases. The tumor suppressor p53 gene is upregulated in response to increased iNOS/NO generation with associated DNA damage (eNOS: endothelial NOS; ERK 1/2: extracellular signal-regulated kinase 1 and 2; PIK: phosphatidylinositol 3-kinase; AKT: protein kinase B; NF-κB: nuclear factor kappa B; iNOS: inducible NOS)

Table 1: NO system and their selected representative interrelationships with IBD associated tumoral development.

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<th>Result</th>
<th>Comment</th>
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<td>Tao et al. (2009)</td>
<td>Cox-2 and iNOS levels were increased in tumors from Hsp70−/− mice compared with Hsp70 WT tumors</td>
<td>Hsp70-deleted mice treated with AOM/DSS develop flat invasive colonic tumors that mimic many histological and molecular features of ulcerative colitis colon cancer</td>
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<td>Erdman et al. (2009)</td>
<td>The presence of Gr-1+ neutrophils and elevated TNF-alpha expression in colon were required for increased iNOS expression and cancer, whereas interleukin-10 (IL-10) down-regulated TNF-alpha and iNOS expression and suppressed cancer.</td>
<td>This study revealed that nitric oxide and TNF-alpha trigger colonic inflammation and carcinogenesis in Helicobacter hepaticus-infected, Rag2-deficient mice.</td>
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<td>Svec et al. (2010)</td>
<td>The specimens of IBD associated colorectal cancer showed upregulated expression of not only iNOS, survivin, c-MYB, Tcf-4, and COX-2.</td>
<td>This markers might serve as early indicators for UC-associated colorectal carcinogenesis.</td>
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<td>Kawanishi et al. (2006)</td>
<td>It was found that nitrative and oxidative DNA lesion products, 8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), were formed and inducible nitric oxide synthase (iNOS) was expressed in epithelial cells and inflammatory cells at the site of carcinogenesis.</td>
<td>It is considered that excessive amounts of reactive nitrogen species produced via iNOS during chronic inflammation may play a key role in carcinogenesis by causing DNA damage.</td>
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<td>Ding et al. (2005)</td>
<td>iNOS, proliferating cell nuclear antigen and p53 protein were expressed in the neoplastic colon epithelium</td>
<td>These results indicate that nitrative DNA damage, as well as oxidative DNA damage, is induced in colon epithelial cells of the IBD mouse model followed by proliferation of these cells, which may contribute to colon carcinogenesis.</td>
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<td>Hofseth et al. (2003)</td>
<td>In noncancerous colon tissues from patients with ulcerative colitis, inducible NO synthase protein levels were positively correlated with p53 serine 15 phosphorylation levels.</td>
<td>This study demonstrates the pivotal role of NO in the induction of cellular stress and the activation of a p53 response pathway during chronic inflammation. iNOS-caused cellular damage.</td>
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<td>Seril et al. (2002)</td>
<td>The number of iNOS positive inflammatory cells in the non-cancerous mucosa of the distal colon was markedly decreased by NAC</td>
<td>This study demonstrated that the antioxidant NAC has the potential to serve as a preventive agent for UC-associated colorectal cancer, possibly via inhibition of cellular proliferation and iNOS-caused cellular damage.</td>
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<td>Hussain et al. (2000)</td>
<td>The colonic nitric oxide synthase-2 activity was higher in UC cases than in non-UC adult controls (p = 0.02). Our data are consistent with the hypothesis that a higher frequency of p53 mutant cells can be generated under oxidative stress in people with UC.</td>
<td>The increased frequency of specific p53 mutated alleles in noncancerous UC colon tissue may confer susceptibility to the development of colorectal cancer in an inflammatory microenvironment.</td>
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<td>Seril et al. (2007)</td>
<td>Nitrotyrosine, -caused protein modification, was detectable by immunohistochemistry in inflammatory cells and epithelial cells of the colon in iNOS+/+ and iNOS-/- mice, and no difference in staining intensity was observed between the two groups. Immunostaining for endothelial NOS (eNOS) was observed in lamina propria macrophages and colonic blood vessels.</td>
<td>This study demonstrated that in the absence of iNOS, other factors, such as eNOS, may play a role in nitrosative stress and UC-associated carcinogenesis in this model system.</td>
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iNOS: inducible nitric oxide synthase; eNOS: endothelial nitric oxide synthase; UC: ulcerative colitis; IBD: inflammatory bowel disease; NAC: N-acetylcysteine
Possibility of predicting 5-year outcomes of ulcerative colitis

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Introduction: Ability to predict unfavorable course of ulcerative colitis (UC) at the beginning of the disease would play a significant role in the management of patients.

Methods: 306 patients with UC were included in a prospective study (55.2% women, age range 16–77). Disease evolution and complications-outcomes of UC were recorded over the 5 years after disease onset. Various demographic, social, hygienic, anamnestic factors, clinical features, treatment terms and methods have been studied as possible risk factors. Stepwise discriminant analysis was used to determine risk factors for unfavorable complicated evolution.

Results: 79 patients (25.8%) were diagnosed with complications-outcomes of UC: colectomy – 3.9%, "inert tube" – 1.6%, rectal stenosis – 1.6%, steroid-dependency – 14.7%, colon cancer – 0%, others – 9.5%. Stepwise discriminant analysis revealed six most important factors which differentiated groups with and without complications: age at the onset, severity of the debut, extraintestinal manifestations, duration of period before a specific treatment and duration up to remission and up to the first exacerbation. The inclusion of these six factors in the formula for the discriminant function allows to predict presence of complications – outcomes after five years with an accuracy of 82.4%.

Discussion/Conclusion: Certain characteristics of the initial period of UC may predict with high accuracy an unfavorable complicated evolution of the disease over the next 5 years.
Immunological features of mouth cavity secretion in children with chronic inflammatory bowel disease

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Aim: To define local oral cavity immunity changes nature in children with investigated pathology.

Materials and methods: 53 patients with ulcerative colitis and 29 ones with Crohn's disease between the ages of 6 and 18 were investigated.

Results and discussion: In patients with inflammatory bowel disease in acute oral secretory immunity, aberration was described, despite the local inflammatory changes absence. This fact reflected mucosa reaction to current pathological process in bowels. In the majority of patients with ulcerative colitis (94.7%) and Crohn's disease (87.5%) IgG elevated level in saliva was detected (p = 0.0001). IgA concentration wasn’t differ from such in healthy children and was elevated only in the half of patients with Crohn's disease (50%) and in 39.5% of children with ulcerative colitis, whereas in 34.2% of patients with ulcerative colitis and 16.7% of children with Crohn's disease its decrease was noted. Secretory IgA indexes were decreased in the majority of children with ulcerative colitis (76.3%) and in the half of patients with Crohn's disease (58.3%). SlgA deficiency increased proportional to disease severity, but during the remission, its level remained below normal in 57% of cases. Revealed local oral cavity immunity factors changes, such as IgG secretion increase against the background of IgA-mediated protection inadequate reaction, IgG/IgA ratio increase, indicated antigenic load enlargement and high risk of immunopathological reactions development in such patients. Saliva cytokine profile studying detected significant increase of IL-1β, TNFα and IL-6 proinflammatory cytokine in acute against the background of normal or reduced IL-4 concentration. IL-10 indexes in saliva were increased in patients with ulcerative colitis (p = 0.00050 and with Crohn's disease (p = 0.04). IL-10 increasing, perhaps, indicates macrophages activation in mucosa along with proinflammatory cytokines level increase.
Predictors of ileostomy effectiveness in complicated Crohn’s disease of the colon

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Introduction: The aim of the study is to improve the surgical treatment results in patients with complicated Crohn’s disease of the colon.

Methods: 43 patients with Crohn’s disease of the colon in the period from January 1998 to July 2009 have undergone the fecal diversion by ileostoma formation, as their first stage of surgical treatment. The following factors analysis has been made: the general somatic state dynamics (index SAI – Severity – Activity – Index [Goebell, 1992] in the clinic’s modification – without counting the amount of stool per day); the colon lesion localization; the dynamics of endoscopic inflammation signs changes of the colon; ultrasound examination data and barium enema data; the severity of perianal lesions.

Results: Out of 43 patients, 41 had progressive improvement of somatic state during the first 2 weeks after the operation. The rest of the factors were studied in 35 (81.3%) out of 43 patients.

In order to find the predictors of ileostomy effectiveness 35 patients with Crohn’s disease of the colon were divided into 2 groups. The first group included 13 patients, in which the remission was achieved. The second group included 22 patients in which the inflammation process in the colon was progressing.

Multifactorial analysis of the treatment results of the second group has been made according to which we found predictors of ileostomy inefficiency: total colon lesion (72.7%); the cobblestone appearance according to radiologic and endoscopic data (68.1% and 63.6%, respectively); the presence of the anal strictures (59.1%); the presence of ulcers and increased vascularization according to ultrasound examination (95.4% and 100%, respectively).

Discussion/Conclusion: With the combination of 3 or more predictors ileostomy formation does not allow to achieve the desired effect and leads to colon resection. With the absence of predictors ileostomy formation and medical therapy in 37.2% of cases leads to remission of the disease.
Epidemiology and outcome of microscopic colitis in northeastern Slovenia

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Introduction: Microscopic colitis (MC) comprising subtypes collagenous (CC) and lymphocytic colitis (LC) is characterised by chronic watery diarrhoea and normal endoscopic findings. There were no data concerning microscopic colitis in Slovenia.

Methods: The aim of our study was to determine the incidence of MC in region of Koroska in northeastern Slovenia and analyze demographics, epidemiological risk factors of patients, natural history and treatment of MC. We performed a retrospective review of medical records of all patients with MC in General Hospital Slovenj Gradec.

Results: In the period from June 2009 to October 2011 MC was diagnosed in 27 patients (16 with CC, seven with LC and four with mixed form). There were 19 women (70.4%) and 8 men (29.6%). Median age at diagnosis was 54 years (range 29–79). The female-male ratio was 2.4:1. The annual incidence rate for MC was approximately 10/100,000. The median interval between onset of symptoms and diagnosis was 27.8 months. Lactose intolerance was diagnosed in 9 out of 14 tested patients (64.3%). Nineteen patients (70.4%) used non-steroidal anti-inflammatory drugs or selective serotonin reuptake inhibitors prior the onset of symptoms. There were no differences in clinical presentation between patients with collagenous and lymphocytic colitis. In three patients (11.1%) symptoms spontaneously resolved without medical treatment and in six patients (22.2%) discontinuing of a possible trigger-drug was enough for clinical improvement. Five patients (18.5%) were treated with loperamide and once (3.7%) budesonide was prescribed. Remission was achieved in all patients with complete data. In 12 cases (44.5%) there were no data about the treatment and further clinical course.

Discussion/Conclusion: Results of our retrospective study shows mild clinical course in patients with MC. The mean annual incidence is 10/100,000. Discontinuing of a possible trigger-drug and use of loperamide is often enough for clinical improvement. Association between lactose intolerance and MC is possible.
Central regulatory role of the transcription factor NFATc2 in cyclosporine A treatment in ulcerative colitis

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Introduction: Cyclosporine A (CsA) is widely used in the treatment of inflammatory diseases. After treatment with CsA an anti-inflammatory effect was observed, resulting in the induction of rapid remission, but this result could not be observed in patients with Crohn’s disease. The aim of our study was to analyse which CsA exerts its therapeutic effect.

Methods: Biopsies from IBD patients were taken and immunofluorescence staining and FACS analysis of apoptosis as well as ELISA analysis of human LPMC were done. NFATc2 ko were used for colitis model analysis.

Results: Isolated LPMC’s from biopsies of patients were examined regarding apoptosis induction upon treatment with CsA for 48 h. Cytometric analysis of apoptotic/necrotic rate revealed a high number of apoptotic cells in UC compared to LPMC’s from CD and control patients. ELISA of supernatants from cultivated LPMC revealed no significant difference regarding the production of IL-2, 4, 5. Significant reduction of the viability of cultivated CD4⁺ T cells from NFATc2 deficient mice in comparison to T cells from control mice could be observed. Due to downregulation of caspase 3 and 9, the induction of apoptosis was elevated in control mice compared to NFATc2 deficient mice. Moreover, naive T cells from NFATc2 deficient and controls mice were isolated and used in colitis transfer model. In contrast to NFATc2 deficient mice, controls showed a significant loss of weight. Furthermore histological score and miniendoscopy revealed a significant lower intestinal inflammation in NFATc2 deficient mice.

Conclusion: Our study points out the central regulatory role of NFATc2. This NFATc2 induced apoptotic pathway is responsible for the clinical efficacy of CsA in the treatment of acute steroid-refractory ulcerative colitis.
Various epithelial markers in patients with ulcerative colitis with or without coexistence of the primary sclerosing cholangitis – Immunohistochemical study

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Introduction: Ulcerative colitis (UC) is inflammatory bowel disease connected with higher occurrence of colorectal carcinoma. The risk is higher in patients with UC and primary sclerosing cholangitis (PSC) and liver transplantation as well. The aim of this study is to evaluate epithelial markers expression of colorectal carcinogenesis in patients with long-term UC with PSC and after liver transplantation for PSC.

Methods: Included were 22 patients with UC after liver transplantation for PSC (OLT), 8 patients with PSC-IBD without liver transplantation, 9 patients with active ulcerative pancolitis, 7 patients with UC in remission and 10 controls. Specimens were analyzed histologically and semi-quantitatively immunohistochemically. For antigen detection we used two-level indirect immunoperoxidase reaction of BCL-2 Oncoprotein, p53 (DakoCytomation), COX-2 (Cayman). For the systemic detection we used immunoperoxidase polymer: Histofine Simple Stain MAX PO (Nichirei) and chromogen 3, 3’ diaminobenzidin (DakoCytomation).

Results: PSC-IBD had statistically significantly higher expression of p53 in the non-dysplatic mucosae as compared to OLT, UCA, UCR and controls (p < 0.01). We also found a statistically significant positive correlation between the incidence of PSC and the expression of P53 (r = 0.4954, p < 0.01). The expression of COX-2 group did not differ PSC-IBD and UCR, but the expression was higher in active colitis (p < 0.01). The expression of bcl-2 was no difference in all groups. Transplant group had significantly lower expression of p53 versus non-transplant group (PSC-IBD) (p < 0.01).

Conclusion: Our work shows that PSC-IBD is associated with higher expression of tumor suppressor gene p53 in non-dysplatic mucosae, and this confirms the increased neoplastic potential PSC-IBD. PSC correlates with the amount of p53 expression. A surprising finding was that a group of UC after liver transplantation for PSC showed low expression of p53 in non-dysplatic mucosae as compared to PSC-IBD and this implies the hypothesis that liver transplantation is unknown mechanism associated with a statistically significant decrease in expression of p53 in the intestinal mucosa.
The influence of vitamin A on the intestinal permeability and a release of cytokines from human in vitro-differentiated macrophages and dendritic cells

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Background and aims: Vitamin A and retinoids are a group of biologically active compounds, which play various roles in the healthy and pathological conditions. They are essential for cell growth, vision and the immune system. Retinoids inflict their biologic activity via retinoic acid receptors (RARs), which bind to retinoic acid response element (RARE) and induce transcription. The aim of this study was to evaluate the potential role of vitamin A and its derivatives on the intestinal permeability and immune response.

Methods: All-trans- (ATRA), 13-cis- (isotretinoin) and 4-oxo-13-cis-retinoic acid (human metabolite of isotretinoin) were used at different concentrations (0.01–5 µg/ml) for all stimulation experiments. Human intestinal cell line Caco2 was grown and stimulated on the transwells. The effect of retinoids on the intestinal permeability was assessed by diffusion of FITC-dextran (10 kDa) across monolayers. Human monocytes were isolated from peripheral mononuclear cells obtained from healthy donors using the monocyte isolation kit (negative selection). From monocytes in vitro-differentiated macrophages (ivMACs) were generated using teflon bags and in vitro-differentiated dendritic cells (ivDCs) by stimulation with IL-4 and GM-CSF. Additionally, in order to confirm findings monocytic/macrophage cell line THP-1 was used. To measure pro- and anti-inflammatory responses cell supernatant was analyzed by multikine ELISA. LPS was used as an inducer of proinflammatory responses.

Results: LPS-stimulated ivMACs and ivDCs pre-treated with retinoids released significantly less TNF (p < 0.0001), IL-6 (p < 0.05), MIP-1α (p < 0.05) and MIP-1β (p < 0.0001). ATRA and its derivatives potentiated LPS-induced release of ICAM-1 from ivDCs (p = 0.001), but not from ivMACs. Retinoids alone induced the release of VEGF (p < 0.05) and IL-10 (p < 0.05) from both ivMACs and ivDCs in a concentration-dependent manner. Consistently, we observed that retinoids inhibited LPS-induced release of IL-6, TNF and MIP-1β in THP-1 cells. In addition ATRA and derivatives induced the release of IL-10 and decreased the production of MIP-1α. Retinoids increased the diffusion of FITC-dextran from apical to the basolateral side of Caco2 monolayers in a concentration-dependent manner.

Conclusions: Vitamin A and its derivatives have distinct effects on different cell types involved in the inflammatory response in the gut. They inhibited the release of proinflammatory cytokines induced by LPS and stimulated the release of anti-inflammatory mediators in macrophages and dendritic cells, alongside with a possible enhancement of intestinal permeability.

Keywords: Retinoids, macrophage, dendritic cell, proinflammatory cytokines, intestinal permeability.
Hypoxia changes mRNA expression levels of SLC transporters in human gastrointestinal tract

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Background and aims: Hypoxia inflicts a broad spectrum of effects on the cellular, organ and systemic levels. In the intestine, hypoxic conditions affect different processes including absorption, metabolism and inflammatory reactions. The aim of this project was to evaluate the influence of hypoxia in high altitudes on the expression levels of solute carrier transporters in human intestine.

Methods: Both serum samples and duodenal biopsies obtained from human subjects (n = 28) exposed to high altitude hypoxia (Capanna Margheritta 4554 MASL) over period of 4 days were analyzed for relevant targets by multikine ELISA as well as by RT-PCR. Results were compared to the serum samples and biopsies taken at 400 MASL (University Hospital Zurich) prior to the exposure to high altitude hypoxia. To verify the findings in \textit{in vitro} system, Caco2 and T84 intestinal epithelial cell lines were incubated in hypoxic chambers in either 0.2% or 1% of oxygen concentration for different time points and analyzed for mRNA and protein levels of implicated targets.

Results: High altitude hypoxia induced the release of IFN-γ, IL-8 and MIP-1β (p < 0.05), which was correlated with the symptoms of mountain sickness and was inhibited by administration of dexamethasone (p < 0.05). Human duodenal biopsies at high altitude expressed significantly lower mRNA levels of nucleoside transporters CNT1 (p < 0.0001) and CNT2 (p = 0.0037), but not ENT2. mRNA expression levels of organic cation transporting polypeptide (OCTN) 2, peptide transporter (PEPT) 1, serotonin transporter (SERT) and organic anion-transporting polypeptide (OATP) 2B1 were significantly lower in hypoxic duodenum (p = 0.02; p = 0.001; p = 0.01 and p = 0.02, respectively). Paired analysis of the samples collected after 4 days of hypoxia showed that the mRNA levels of CNT1 further decreased, while the mRNA expression of CNT2, OATP2B1 and SERT remained at the same level. mRNA levels of OCTN2, PEPT1, and ENT2 returned to normal levels on the 4th day at high altitude, indicating the involvement of adaptive mechanisms in the transcriptional control of these transporters. These changes were accompanied by a minor increase of glucose transporter (GLUT) 1 mRNA. In addition we detected a decrease in both TNF (p = 0.01) and ICAM (p = 0.02) mRNAs and an increase in IL-8 (p = 0.09) mRNA levels. In both Caco2 and T84 intestinal epithelial cells cultivated in hypoxic chambers we observed a time-dependent decrease of CNT1, OATP2B1 and SERT mRNAs, whereas the levels of OCTN1 remained unchanged. The decreased mRNA expression levels of SERT, ENT2, OCTN2, OCTN2B1 and CNT1 were already detected 2 hours after induction of hypoxia, suggesting early and specific transcriptional repression of these target genes triggered by oxygen deprivation. In T84 cells hypoxia induced protein expression levels of GLUT-1 and phosphorylation of Akt and decreased protein levels of ENT2 and OCTN2.
Conclusions: Oxygen deprivation triggers the release of proinflammatory mediators on a systemic level and in parallel represses the transcription of SLC transporters’ genes in human intestine. This can have consequences for intestinal absorption, and can provide clues about the transcriptional regulation of these transporters, which is perturbed in IBD intestine.

Keywords: SLC transporter, hypoxia, intestine, transcriptional regulation.

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Fc gamma receptors modulate the inhibitory efficacy of infliximab in blocking TNF-mediated responses in blood and intestine of IBD patients

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Background and aims: Tumor necrosis factor (TNF) is an important cytokine in the pathogenesis of inflammatory bowel disease (IBD). Anti-TNF drugs have been implemented in IBD therapy. The efficacies of those drugs differ among individuals and they have not been tested in different tissues. The aim of this study was to evaluate their efficacies in ex vivo-treated peripheral blood and intestine of both IBD patients and healthy individuals.

Methods: Peripheral blood obtained from healthy donors and IBD patients was used for stimulation experiments with anti-TNF drugs. Peripheral blood mononuclear cells (PBMCs) were isolated and the efficacies of anti-TNFs were assessed by RT-PCR and ELISA. Expression levels of Fc receptors were examined by Western blotting and RT-PCR. To determine the activation of Fc receptors we measured the production of GM-CSF and CCL-2 mRNAs and phospho-tyrosine signal in THP-1 cells. The expression levels of Fc receptors in human intestinal tissues and in PBMCs were determined by RT-PCR and Western blotting.

Results: Both adalimumab (ADA) and infliximab (IFX) displayed significant limitations in blocking TNF-mediated responses in ex vivo-treated peripheral blood from healthy donors as compared to certolizumab-pegol (CZP; p < 0.001). ADA had significantly lower efficacy in PBMCs as compared to THP-1 cells (p < 0.001), which was correlated with the increased expression levels of both low affinity Fc receptors CD16 and CD32. IFX, but no other anti-TNFs tested was less effective in peripheral blood of IBD patients as compared to healthy controls (p < 0.05) when assessed by measuring the changes on mRNA levels of IL-8, TNF and ICAM as well as plasma release of IL-8 (p < 0.05). These differences were correlated with an increase in mRNA levels of both low affinity Fc receptors CD16 and CD32 in PBMCs from IBD patients as compared to healthy individuals. In THP-1 cells IFX either alone or in complex with TNF, was more potent in activating Fc receptors as measured by the production of GM-CSF and CCL-2 mRNA (p < 0.05). IFX/TNF complexes induced IL-8 mRNA production in THP-1 cells (p < 0.05), which was accompanied by detection of distinct phospho-tyrosine signals. Increased colonic mRNA (p < 0.05) and protein (p < 0.001) expression levels of CD64 as well as mRNA levels of CD16 (p < 0.001) were detected in inflamed tissues of IFX-non-responders as compared to IFX responders. These changes were correlated with elevated mRNA levels of cytokines regulating the expression of this receptor, namely GM-CSF (p < 0.01) and IFN-γ (p < 0.0001) as well as IL-8 (p < 0.0001) and IL-6 (p < 0.05).

Conclusions: Fc receptors modulate the efficacy of IFX both ex- and in vivo. These observations will help to optimize individual anti-TNF strategy in IBD patients with elevated systemic and local levels of Fc receptors.

Keywords: Fc receptor, anti-TNFs, peripheral blood.
Analysis of the incidental diagnosis of inflammatory bowel disease made during the Scottish bowel cancer screening programme

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Introduction: The Scottish bowel screening programme (SBSP) has been running since March 2008 in patients aged between 50 and 74. The aim of this study was to quantify the number of new cases of inflammatory bowel disease (IBD) diagnosed as part of the SBSP in South East Scotland. The progression of these patients was also assessed.

Methods: All the patients with histologically confirmed diagnosis of IBD were identified during the first three years of SBSP in South East Scotland. Further information like symptoms at onset, risk factors, disease severity and initial treatment was collated. The patients' progress following diagnosis was also assessed.

Results: 51 (1.4%) patients with IBD were diagnosed out of 3655 procedures performed between June 2008 and April 2011. Of these, 12 (0.3%) patients had previous diagnosis of IBD and were excluded from study. In new patients of IBD (n = 39), significantly more males 30 (77%), (mean age – 63), were diagnosed with IBD than females (9.23%), (mean age – 67). 12 (30%) patients were diagnosed with CD, 16 (41%) had UC and 11 (28.2%) had IBD unclassified (IBDU).

26 (67%) patients were symptomatic at the time of diagnosis with a mean Mayo score of 2.4 for ulcerative colitis and a mean Harvey Bradshaw score of 1.4 for Crohn's colitis group.

34 (87%) patients were in remission through out the follow up period (6–30 months). 9 (23%) of these had no treatment, 19 (48.4%) had oral or topical mesalazine, 4 (10%) had oral steroids while 3 (7.6%) patients required both oral steroids and mesalazine. 5 patients were unresponsive to initial therapy (2 – CD, 1 – UC, 2 – IBDU) and required azathioprine (n = 3), oral steroids (n = 1) or methotrexate (n = 1).

Discussion/Conclusion: In this cohort of 3655 patients attending for bowel cancer screening colonoscopy, IBD was diagnosed in 1.1% of patients. There was a preponderance of male patients. When assessed the majority of patients had previous symptoms and following diagnosis their IBD followed a benign course.
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Dealing with our “In-vironment”: New Aspects in IBD Pathogenesis and Therapy

May 4 – 5, 2012
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