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Gut-Liver Interactions: Basic and Clinical Concepts

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Abstracts of Invited Lectures
Poster Abstracts

Falk Symposium 146

GUT-LIVER INTERACTIONS:
BASIC AND CLINICAL CONCEPTS

Innsbruck (Austria)
March 11-12, 2005

Scientific Organization:
R.S. Blumberg, Boston (USA)
A. Gangl, Vienna (Austria)
M.P. Manns, Hannover (Germany)
H. Tilg, Hall/Tirol (Austria)
M. Zeitz, Berlin (Germany)
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Genetics and immunology in inflammatory bowel diseases (IBD)
Emerging data suggest that IBD comprises not two disorders but a heterogeneous family of inflammatory disorders in which the specific clinical manifestations of disease in any individual are determined by the interaction of genetic and environmental factors. A genetic model for IBD phenotype has been proposed in which an individual’s disease characteristics are determined by the interaction of both susceptibility and modifying genes. In this model, susceptibility gene mutations influence the development of distinct clinical phenotypes. Mutations in other, modifier genes, do not initiate disease, but once disease is present, will act to influence specific features of disease phenotype, for example disease penetrance, progression, complications, and response to treatment.

Definitive gene associations have been established for the CARD15 gene on chromosome 16 and the OCTN cluster on chromosome 5q (IBD5), both conferring increased risk of CD. Significant associations have been reported for additional genes, including DLG5, MDR1 and TLR4. All the clinically recognized forms of CD can be found in patients with and without CARD15 mutations. Nevertheless, a consistent pattern is that CARD15 variants are associated with younger age at onset, presence of ileal involvement, and a tendency to develop strictures. Data are still conflicting as to whether CARD15 carriage is associated with a more severe disease course and risk for early surgery. One potential explanation for the association with ileal disease is the prominent expression of CARD15 in Paneth cells typically found in the terminal ileum. The results from genotype-phenotype analyses using other IBD susceptibility genes are still preliminary. A study reported that the IBD5 risk haplotype was specifically associated with perianal CD, with the greatest relative risk seen in homozygous individuals. The question arises whether the OCTN variants are associated with the same phenotype.

Genes in the HLA region on chromosome 6 may have a greater role for modifying IBD phenotype than on determining overall disease susceptibility. Within UC clinical subgroups, the uncommon DRB1*0103 allele is associated with both extensive disease and severe disease as judged by the need for colectomy. A number of genetic associations have also been described with the extra-intestinal manifestations of IBD. A lack of reproducibility has challenged many of the other reported genotype phenotype associations in IBD. These include the association in UC between allele 2 of the (IL)-1ra gene and extensive colitis, the need for colectomy, and the development of pouchitis after ileal-pouch anastomosis.

Incorporating comprehensive IBD phenotyping in linkage studies may provide crucial insights into pathophysiologic mechanisms of risk alleles. Unfortunately, with the exception of the association between CARD15 and ileal disease location, the inconsistency of data from genotype/phenotype studies in IBD has limited their usefulness. Problems relate to difficulties in the clinical classification and definitions of disease, inconstancy of some phenotypes with time, lack of consideration of confounding factors on disease complications such as smoking, scarcity of population-based studies and genetic heterogeneity.
The role of cytokines in the pathogenesis of experimental and human inflammatory diseases of the mucosal immune system

W. Strober, M.D.
National Institutes of Health, Mucosal Immunity Section, Bethesda, MD, USA

Recent advances in our knowledge of the immunopathogenesis of inflammatory bowel disease (IBD) now make it possible to design more specific cytokine-based therapies for these diseases.

With respect to Crohn’s disease, it is now well documented that Crohn’s disease is associated with a Th1 T cell-mediated inflammatory response and, as such, it is driven by the “master” Th1 cytokine(s), IL-12 (and possibly IL-23). Previous studies initially performed in the context of hapten-induced colitis (TNBS-colitis) and then performed in the context of many other experimental colitides established that anti-IL-12 was an effective treatment of experimental mucosal inflammation due to a Th1-driven process. In addition, it was shown that the basis of this therapeutic effect was the ability of anti-IL-12 to induce apoptosis of the effector T cells (Review in Nature Immunology Reviews).

On this basis, we participated in and energized the development of a fully human (monoclonal) anti-IL-12 (anti-p40) by Wyeth Pharmaceuticals for treatment of patients with Crohn’s disease and possibly other Th1-mediated diseases. When this goal was ultimately attained, we and other investigators worked with Wyeth to plan and execute a (phase I-II) double-blind, placebo-controlled trial of anti-IL-12 in 79 patients with active Crohn’s disease at NIH and other medical centers. In this study patients were randomly assigned to receive seven weekly subcutaneous injections of 1 or 3 mg/kg of anti-IL-12 or placebo with either a four week interval between the first and second injection (Cohort 1) or no interruption (Cohort 2). Patients in Cohort 2 receiving 3 mg/kg antibody showed significant differences in response rates (highest response rate 75% at week 6) and remission rates (highest remission rate 50% at week 19) compared to patients receiving placebo, whereas the high clinical response and remission rates in Cohort 1 patients receiving antibody were not significantly different from the placebo group. The effects of antibody treatment were durable and adverse events were comparable to placebo except for mild local injection reactions.

In patients studied at the NIH, the effect of treatment on cytokine production by lamina propria cells extracted from biopsy specimens were studied. Anti-IL-12 treatment was accompanied by significant decreases in IL-12, IFN-γ, IL-17 and TNF-α production, but no effect on IL-4 or IL-10 production.

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These studies show that monoclonal anti-IL-12 (anti-p40) is an effective treatment of active Crohn’s disease and pave the way for a phase III trial of this agent in a larger patient group. In addition, provides solid evidence that a Th1 process is final common T cell pathway underlying the inflammation present in Crohn’s disease.

With respect to ulcerative colitis (UC), we are also making progress in devising an approach to therapy based on a primary immunologic abnormality. In particular, we have recently reported that UC is associated with increased IL-13 secretion by a class of cells known as NKT cells. In addition, evidence has been obtained that the IL-13 enhances the ability of the NKT cells to act as cytolytic cells for epithelial cells. This, plus the fact that IL-13 has direct toxicity for epithelial cells, may explain why this form of IBD is marked by superficial inflammation and epithelial ulceration. To determine if IL-13 has the same relation to UC as IL-12 has to CD (as discussed above) will require a large clinical study involving the treatment of patients with an IL-13 inhibitor. However, the fact that an murine model of ulcerative colitis, oxazolone colitis, is also due to IL-13 secretion by NKT cells, and, more importantly, is prevented by IL-13 inhibitors, bodes well for the possibility that such inhibitors will also be efficacious in UC.
Dendritic cells: Key players in IBD

Dr. A. Kaser
Universitätsklinik Innsbruck, Innsbruck, Austria

Dendritic cells (DC) are antigen-presenting cells that act as sentinels, acquiring antigen and transporting it to lymphoid tissue where they have the unique ability to activate naïve T cells. Therefore, DC are considered to be pivotal for maintaining the delicate balance between tolerance toward the commensal microbial flora on the one hand and active immunity towards invading pathogens on the other. This presentation will cover several key aspects of dendritic cell biology in the mucosa and its relevance for inflammatory bowel disease.

Mucosal DC continuously traffic through and survey the intestinal compartment, with several chemokine/receptor pairs orchestrating the selective recruitment of DC. Thus DC get in close contact to intestinal luminal contents and can sample antigens from the intestinal lumen. Microbial products recognized by pattern recognition receptors as well as cytokines in the mucosal micro-environment modulate DC function and contribute to their ability to promote tolerance or generate adaptive immune responses. Of note is that DC in patients with inflammatory bowel disease demonstrate a mature, activated phenotype. An additional function of intestinal (Peyer’s patch) dendritic cells is their capacity to imprint gut-homing specificity on T cells, thus licensing effector/memory cells to access the site of their cognate antigen. Most importantly, blocking DC function, in particular DC-T cell interaction, could ameliorate disease in animal experimental models of colitis.

In conclusion, DC might have a unique function in a disease in which the antigenic abundance of the commensal flora is considered a major contributor to the disease process.
Lipids and IBD: Microsomal triglyceride transfer protein – The new dimension?

Stephanie Betz, Arthur Kaser, Takashi Nagaishi, Masaru Yoshida, Mahmoud Hussain, Bryan vander Lugt, Mark Exley, Richard S. Blumberg
GI Division, Brigham and Women's Hospital, Boston, MA, USA

CD1d is a major histocompatibility complex (MHC) class I-related molecule that functions in glycolipid antigen presentation to distinct subsets of T cells that express natural killer cell receptors and the invariant T cell receptor-α chain (invariant NKT cells). CD1d is expressed widely by hematopoietic cells, especially dendritic cells and B cells, and nonprofessional antigen-presenting cells such as hepatocytes and intestinal epithelial cells. The acquisition of glycolipid antigens of CD1d occurs, in part, in endosomes through the function of resident lipid transfer proteins, namely saposins. The acquisition of glycolipid antigens in the endoplasmic reticulum, where CD1d first forms, is unknown. It has been assumed that the initial formation of mature and folded CD1d protein requires the acquisition of a glycolipid antigen in the endoplasmic reticulum akin to the acquisition of peptides by MHC class I molecules. We have shown that microsomal triglyceride transfer protein (MTP), a protein that resides in the endoplasmic reticulum of hepatocytes and intestinal epithelial cells and essential for the lipidation of apolipoprotein B, associates with CD1d in hepatocytes. Hepatocytes from animals in which mttp (the gene encoding MTP) has been conditionally deleted, and IECs in which mttp gene products have been silenced, are unable to activate invariant NKT cells. Conditional deletion of the mttp in hepatocytes is associated with the redistribution of CD1d expression, and MTP-deleted mice are resistant to the immunopathologies associated with invariant NKT cell-mediated hepatitis (initiated by the injection of a model glycolipid antigen that activates invariant NKT cells, α-galactosylceramide) and colitis (induced by the administration of the hapten, oxazolone). In addition, we have also observed that MTP is expressed beyond intestinal epithelial cells and hepatocytes. Using Western blotting and semiquantitative RT-PCR, we have observed expression of MTP in a wide variety of hematopoietic cells (including dendritic cells and B cells) and parenchymal tissues beyond the liver and the intestine. Using gene silencing, chemical inhibition with small molecule inhibitors that block MTP lipidation function or gene deletion of MTP, we also have found that functional expression of MTP extends to mouse and human professional antigen presenting cells. Furthermore, we have shown that MTP can directly transfer lipids to recombinant CD1d in vitro. Taken together, these studies suggest that MTP acts upstream of the saposins and functions as a chaperone by loading endogenous lipids in the nascent CD1d groove. Furthermore, our studies suggest that a small molecule inhibitor can be used to modulate the activity of NKT cells. These studies indicate that CD1d-regulating function of MTP in the endoplasmic reticulum is complementary to that of the saposins in endosomes in vivo. Finally, these studies show how proteins that have previously been associated with absorption of lipids are also involved in regulating the immune response.
The intestinal epithelium and IBD

Daniel K. Podolsky, M.D.
Chief, GI Unit Massachusetts General Hospital, Mallinckrodt Professor of Medicine, Harvard Medical School, Boston, MA, USA

Accumulating evidence underscores the important role that the epithelium plays in both pathogenesis and pathophysiology of Inflammatory Bowel Diseases. Barrier function was the first epithelial property to be recognized for its relevance to inflammatory bowel disease. Studies more than a decade ago suggested that functional alterations in this barrier function (“permeability”) could be found not only in patients with Crohn’s disease but in some first degree relatives. For the past decade tremendous additional molecular detail of the structures necessary to form tight junctions which are the single most important determinant of barrier function have emerged. While no primary alterations in these structural proteins have yet been identified, their integrity and with it paracellular transport are clearly modulated by a variety of inflammatory mediators. Barrier function is now also recognized to result from structures other than the tight junction. These include the pre-epithelial compartment comprised of trefoil peptides and other products of goblet cell populations.

In recent years the new concepts of the role of epithelium as a living barrier suggest other key relevant to inflammatory bowel disease have emerged. The entire epithelial cell can be characterized as a sensor, mediating interactions with complex luminal milieu at its apical surface and with the capacity for “read-out” in the form of basolateral secretion of various regulatory peptides which evoke cell recruitment and modulation of the underlying lamina propria. This global perspective on the role of the epithelial cell as an integrated unit follows from accumulating evidence of the participation of the epithelial cells in both adaptive and innate immune mechanisms. Many studies have demonstrated the ability of intestinal epithelial cells to serve as non classical antigen presenting cells (APCs), a capability which is likely facilitated in the context of IBD by the induced expression of MHC class II. Notwithstanding these properties, studies in the past few years suggest that the epithelium may play an even more important role in innate immune mechanisms. Thus these cells expressed both the cell surface family of pattern molecule receptors designated TLR (Toll-like receptors) as well as cytoplasmic pattern recognition receptors of the Nod family. It is noteworthy that despite early assumption that the Nod2 protein encoded by the IBD1 locus was exclusively expressed in monocyctic lineages overall there is a greater expression within the epithelial compartment. The latter may be further enhanced in the context of IBD by TNF and perhaps other cytokines. The role of the epithelium as integrated sensory unit converges with this evolved understanding of its innate immune capabilities and the demonstration that the IBD5 locus encodes a cation transporter which is largely if not exclusively expressed within the epithelium and may be responsible for cellular uptake of pattern molecules derived from luminal flora.
Finally, the epithelium may determine the outcome of the interaction between the mucosa and luminal flora which increasingly appears to be key determining factor in the initiation and the perpetuation of the IBD. Thus the epithelium is able to effect some cell killing of low level invasive bacteria and indeed within the specialized compartment of Paneth cells as a source of cryptdins and defensins which have direct bactericidal actions.

In summary, accumulating evidence from *in vitro* and *in vivo* studies increasingly point to dysfunction in the epithelium as central to the pathogenesis of IBD and in some circumstances sufficient for development of IBD.
Evidence-based treatment in IBD
Evidence-based therapy with aminosalicylates

Univ.-Prof. Dr. Walter Reinisch
Universitätsklinik für Innere Medizin IV, Abteilung Gastroenterologie und Hepatologie, AKH Wien, Austria

Sulfasalazine and 5-aminosalicylic acid (5-ASA, mesalazine) represented major advances in the treatment of ulcerative colitis (UC). Over decades 5-ASA therapy still remains first line standard in the induction and maintenance of remission for mild to moderate UC. A meta-analysis demonstrated that the different, available oral formulations of aminosalicylates showed similar plasma pharmacokinetics suggesting comparable clinical efficacy in extensive disease. However, mesalazine is associated with a more favourable toxicity profile compared with sulfasalazine. Rectal 5-ASA formulation are most efficacious in distal UC. Preliminary evidence from uncontrolled trial emerges that 5-ASA appears to attenuate the rate of progression of colorectal cancer in patients with UC.

Initial published trials lead to the conclusion that oral aminosalicylates are also an effective treatment for mild to moderate ileal, ileocolic or colonic Crohn’s disease (CD). However, in a recent meta-analysis a mean reduction of the CDAI from baseline of −63 points for mesalazine, compared to −45 points for placebo (p = 0.04) was shown, which confirms a superiority of mesalazine 4g/day to placebo, but is of debatable clinical significance. Similarly, data from meta-analyses including studies of better quality does not appear to reveal efficacy of mesalazine for maintenance of medically induced remission, even if suggested by the first placebo-controlled studies. Patients with localized ileal Crohn’s disease might, however, benefit from this treatment. The effectiveness of sulfasalazine is not established in the maintenance treatment of CD. For postsurgical maintenance of remission a benefit of mesalazin is suggested for patients with ileal disease.

Correspondence:

Univ.-Prof. Dr. Walter Reinisch
Universitätsklinik für Innere Medizin IV
Abteilung Gastroenterologie und Hepatologie
AKH Wien
Währinger Gürtel 18-20
A-1090 Wien
Austria
Practical use of steroids in IBD

Geert D’Haens M.D., Ph.D.
Univ. Ziekenhuis Gasthuisberg, Department of Gastroenterology, Leuven, Belgium

Corticosteroids remain an important treatment modality for moderate to severe forms of both Crohn’s disease and ulcerative colitis. Their position in the therapeutic armamentarium is somewhat different for both conditions.

In Crohn’s disease, 5-ASA preparations are relatively weak to induce remission of active disease. The majority of patients will need corticosteroids, preferably topically acting agents such as budesonide. Whereas the agent ‘Entocort’ (Budesonide CIR) was specifically designed for ileocecal Crohn’s disease, the other agent Budenofalk has been used effectively for all forms of Crohn’s disease. The side effect profile of both agents is much more favourable than that of classic, systemic corticosteroids. Nonetheless, all adverse events reported with steroid agents can develop, but generally they are less severe and definitely occur less frequently. In particular bone loss is significantly reduced with topical steroids when compared with systemic agents.

The maintenance benefits of corticosteroids, including the topical agents, are limited. A further ‘relapse’ of Crohn’s disease can be delayed to a certain extent, but at the end of one year the incidence of relapses is similar to the one observed with placebo treatment. As a matter of fact, maintenance therapy with corticosteroids is unacceptable in the majority of patients, given the availability of potent immunomodulatory and steroids sparing agents such as azathioprine, MTX and infliximab. Patients who despite these medications continue to be dependent on steroids can safely be ‘switched’ to topical steroids, although surgical resection of the diseased (and often narrowed) bowel segments then needs to be seriously considered.

In ulcerative colitis, many more patients can be treated successfully with 5-ASA agents alone (oral and/or topical). Corticosteroids are only needed in more severe disease attacks. For left-sided disease, a topical (enema) preparation with budesonide 2 mg/100 ml has been developed. More refractory patients can even be treated with a combination enema of 5-ASA and a topical steroid such as beclomethasone dipropionate. New galenic forms for ‘colonic release’ of budesonide are currently under study. Patients requiring treatment with systemic steroids should be tapered off this medication as soon as the disease is in remission; in case of steroid dependency, immunomodulators should be initiated. Patients who are refractory to systemic steroids will be treated with intravenous cyclosporine, infliximab or even proctocolectomy.
Immunosuppressive drugs: Clinical use and mechanism of action

Markus F. Neurath
Innere Medizin I, Universitätsklinikum Mainz, Germany

Inflammatory bowel diseases (IBD) are mediated by an uncontrolled activation of the mucosal immune system thus explaining the high clinical value of immunosuppressive therapy in IBD patients. Recent studies showed that antibodies against proinflammatory cytokines such as anti-IL-12, anti-IL-6R and anti-TNF antibodies suppress established intestinal inflammation by inducing T cell apoptosis. For instance, anti-TNF antibodies have been shown to bind to membrane bound TNF on the T cell surface followed by the induction of T cell apoptosis. These findings have important implications for understanding therapeutic approaches for IBD patients.

Azathioprine and its metabolite 6-mercaptopurine (6-MP) are established immunosuppressive drugs in IBD patients that are frequently used in steroid-dependent or steroid-refractory cases. Both have been shown to induce T cell apoptosis in IBD patients. Mechanistically, azathioprine generated 6-thio-GTP which binds to and inactivates the small GTPase Rac1 in T cells thereby causing a mitochondrial pathway of T cell apoptosis. Apoptosis induction required costimulation with CD28 and was mediated by specific blockade of Rac1 activation via binding of azathioprine generated 6-ThioGTP to Rac1 instead of GTP. The activation of Rac1 target genes such as MEK, NF-kappaB and bcl-xL was suppressed by azathioprine leading to a mitochondrial pathway of apoptosis. Azathioprine thus converts a costimulatory signal into an apoptotic signal by modulating Rac1 activity. These findings explain the immunosuppressive effects of azathioprine and suggest that 6-Thio-GTP derivates may be useful as potent immunosuppressive agents in autoimmune diseases and organ transplantation.

Furthermore, cyclosporin-A that is used during acute flares in patients with ulcerative colitis can modulate T cell survival and cytokine production. Specifically, it binds to the immunophilin CypA in T cells thereby blocking activation of Itk and NFAT. This is followed by blockade of Th2 cytokine production in T lymphocytes.

Collectively, these data imply that T cell cytokine production and apoptosis are key targets for designing novel therapeutic strategies in IBD patients.
Biologicals: Old and new ones

Paul Rutgeerts
University of Leuven, Belgium

The advent of the anti TNF agent infliximab has dramatically changed our concept of treating refractory IBD, particularly Crohn’s disease. Although infliximab has proven to induce clinical response and remission with rapid onset, to spare steroids, to improve perianal disease and to increase quality of life, there is a considerable unmet medical need in both Crohn’s disease and ulcerative colitis. Still 30% of patients with refractory Crohn’s disease do not respond to infliximab treatment. Moreover, the long-term use of this drug is associated with immunogenicity, which interferes with efficacy, and with the risk for infectious complications. In ulcerative colitis the role of anti TNF agents is still not fully established and the long term outcome of colectomy as an alternative to continuing medical treatment is far from optimal. Therefore, the quest for novel biological treatments goes on.

Several strategies are followed in drug development to improve the efficacy and tolerability of biological agents. First, progress in protein engineering has resulted in the elimination of immunogenic non-human peptide sequences from anti-human antibodies, a technique known as humanization. Third generation, humanized antibodies (95% human) and fourth generation, fully (100%) human antibodies, are usually associated with less immunogenicity as compared to chimeric (75% human) monoclonals such as infliximab. Also, subcutaneous injection eliminating the need for in-hospital infusions is the preferred method of drug administration for novel biologicals. Finally, pathways in the immune reaction not directly involving TNF inhibition, are being targeted.

For Crohn’s disease selective inhibition of adhesion molecules is an effective maintenance therapy and antibodies to the alpha4 integrins will be introduced into the clinic soon. Other promising approaches in the treatment of Crohn’s disease are anti-IL-12 strategies, anti-IL-6 R antibodies and GM-CSF. The efficacy and safety of the latter drugs need to be proven. Moreover it is not clear how these drugs will be positioned in the strategies of Crohn’s disease treatment.

In ulcerative colitis the unmet need is in the treatment of severe refractory UC and chronic active disease. Candidate drugs to treat the former severe problem are infliximab and antibodies to CD 3. These should be an alternative to cyclosporin and avoid colectomy. Also antibodies to CD 25 are under study but data are controversial. Therapies using anti-inflammatory cytokines produced by recombinant technology have largely failed and this suggests that probably the type of cell activated is more important in IBD than the balance of cytokines in the tissue.

There is a great need for more simple therapies. Antibody treatments have allowed us to define the exact targets for our therapies but the development of small oral molecules blocking the same pathways would allow improved therapy.

Finally we need to investigate whether biological therapies change the long-term outcome of Crohn’s disease and ulcerative colitis by inducing complete bowel healing resulting in decreased rate of complications, hospitalizations, surgeries and even mortality.
Difficult cases in inflammatory bowel diseases (IBD)
Case presentation: Ulcerative colitis (UC)

H. Lochs
Charité Universitätskliniken, Gastroenterologie/Hepatologie, Berlin, Germany

(Discussion together with W. Petritsch, Graz)
Case presentation: Crohn's disease (CD) (and surgery)

S. Schreiber
Universitätsklinikum Schleswig-Holstein, Innere Medizin I, Kiel, Germany

(Discussion together with V. Annese, San Giovanni Rotondo and J. Tepel, Kiel)
Management of perianal and fistulizing disease

Brian G. Feagan MD
University of Western Ontario, Robarts Research Institute, London, Ontario, Canada

The natural history of fistulizing Crohn's disease has been established in population-based cohort studies. Approximately one-third of patients develop fistulizing disease at some point. The majority of these (2/3) occur in the perianal area and are simple with a single orifice. The prognosis for these cases is usually good; many will resolve spontaneously or with minimal medical or surgical treatment. However, more severe variants are associated with considerable disability and are a formidable problem for gastroenterologists.

The etiology of fistulization is poorly understood. In the small bowel fistulization frequently occurs proximal to a stenotic segment. In the anal canal tracts arise from anal crypts or from deep ulcers. The majority of patients with rectal involvement ultimately will develop a fistula. The transmural nature of Crohn's disease, bacteria and mechanical pressure all likely contribute to the pathology.

From a clinical perspective a combined medical and surgical approach is the conventional method of treatment. The Parks Classification is a useful anatomical system of differentiating between simple and complex fistulas. Fistulas that involve the internal and external sphincters are complex and more difficult to treat. Several possibilities exist to define anatomy including examination under anesthesia, MRI and endoscopic ultrasound. Each of these modalities is relatively sensitive and specific as a single test, however a prospective study by Sandborn and colleagues indicated that the combination of two of these tests yielded an optimum result. Due to the high degree of operator dependence with examination under anesthesia and rectal ultrasonography, clinicians must develop a diagnostic scheme that takes into account local resources. The widespread availability of MRI scanning has improved the diagnostic/therapeutic algorithm. The goal of diagnostic assessment should be to accurately define the anatomy and to exclude the presence of an abscess. Assessment of the extent and severity of luminal disease is also necessary as a component of a comprehensive approach to the problem. Barium studies and colonoscopy are usually indicated.

Once the anatomy has been defined definitive action can be undertaken. Abscesses should be drained and luminal disuse treated. Simple fistulas can be treated medically with antibiotics or excised. Complex fistulas require a combined medical-surgical approach with placement of Setons and initiation of immunosuppressive drug therapy.

No randomized controlled trials have been performed to evaluate the efficacy of the purine antimetabolites in fistulizing disease. A subgroup analysis of Present's original trial of 6-MP suggested a beneficial effect, however the lack of a standardized measure of outcome makes these data difficult to interpret. Observational studies suggest that these agents are beneficial. Limited data also indicate that methotrexate may be effective.

Given the lack of definitive data for the broad-spectrum immunosuppressives my personal view is that infliximab is the primary medical therapy for complex fistulizing disease. Data from two large randomized controlled trials support this position. In an initial study Present and colleagues demonstrated that the administration of infliximab 5 mg/kg (Weeks 0, 2 and 6) closed 50 percent of draining fistulas in 68% of patients compared with 13% of those who received placebo. Subsequently, Sands and
colleagues showed that continued administration of infliximab every eight weeks was more effective than a single course of therapy. It is important to recognize however, that infliximab is not a monotherapy. Co-administration of an antimetabolite (azathioprine, 6-mercaptopurine, methotrexate) is required to prevent the development of antibodies to infliximab. Given this situation high-quality data that evaluate the relative efficacy of these drugs is badly needed.

The only other drug that has demonstrated a benefit in a randomized controlled trial is tacrolimus. Sandborn and colleagues evaluated 42 patients that had failed to respond to other forms of treatment including infliximab. A benefit was shown for the proportion of patients who closed 50% of or more draining orifices, however no significant effect was demonstrated for complete remission. The dose of tacrolimus utilized (0.2 mg/kg) was associated with a clinically important increase in the serum creatinine concentration. Accordingly, administration of tacrolimus on a prolonged basis is probably not safe. Evaluation of lower doses of this drug in combination with infliximab is a subject for further studies.

Once a patient has begun long-term immunosuppression re-evaluation of the response to treatment is required. MRI studies have demonstrated that re-epithelialization of the external orifice does not directly correlate with obliteration of the tract. Although adjustment of treatment regimens according to MRI response has not been rigorously evaluated, this appears to be a sensible approach to the management of this chronic disease. Patients who fail aggressive medical therapy may require ileostomy and or proctocolectomy. Hopefully with continued improvement of our treatment regimens this outcome will occur less frequently in the future.

Selected References:


Management of extraintestinal disease in IBD

Jürgen Schölmerich
Klinikum der Universität Regensburg, Innere Medizin I, Regensburg, Germany

In addition to the classical symptoms diarrhea, pain and weigh loss, numerous extraintestinal symptoms can be found in IBD, which often determine the clinical course to a significant extent and are sometimes difficult to recognize or to treat.

Extraintestinal symptoms can be divided into two groups: extraintestinal manifestations (EM) and extraintestinal complications (EC). Those are separated by the fact that the pathogenesis of EM is mostly unknown in analogy to the underlying diseases. While that of EC is due to the disturbed function of the gut and can be easily explained. EM mostly affect external organs such as skin, eyes and joins and are easy to recognize. In contrast EC, in particular deficiency states have a more subclinical course and are often diagnosed only late.

While the treatment of EM is anti-inflammatory in nature in analogy to the primary disorder EC can often be treated causally, i.e. by substitution of micronutrients or influencing intestinal function.

Beside joints, skin and eyes EM have been described on the heart, the pancreas, the liver, the kidneys, the brain and in particular the lungs. In total about two third of all patients with IBD present with EM during the course of their disease. The frequency is higher with colonic involvement. Most of these symptoms do not need separate treatment or treatment at all, since their activity waxes and wanes with the underlying disorder. Notable exceptions are pyoderma gangraenosum, primary sclerosing cholangitis and spondylarthropathy among others. If treatment is necessary anti-inflammatory medications, in particular glucocorticosteroids, more effective immunosuppressants and a number of other principles can be used. It should be mentioned here that NSAID should be avoided since they can activate the underlying disorder. Eye disorders, in particular iridocyclitis and uveitis need to be treated locally with steroids since delayed improvement can lead to permanent lesions. Pyoderma gangraenosum needs additional topical treatment. Azathioprin and cyclosporin A can be used successfully. Ankylosing spondylitis can be treated very effectively with Infliximab. Severe pulmonary abnormalities need to be treated with steroids and eventually with added immunosuppression. Primary sclerosing cholangitis is treated with ursodeoxyxolic acid, substitution of fat soluble vitamins has been recommended. Finally liver transplantation needs to be considered.

Extraintestinal complications are based in particular on an excess or a lack of exogenous or endogenous substances in particular body compartments. Deficiency syndromes regarding vitamins, trace elements, proteins and water are of importance as well as an increased absorption of bilirubin and oxalic acid as cause of biliary and kidney stones. Deficiency syndromes can be severe and can lead to osteomalacia, night blindness, anaemia, growth retardation, infertility, edema and other problems.
These complications are in particular found in Crohn's disease, in ulcerative colitis mainly iron deficiency has been described. The treatment of deficiency syndromes is for obvious reasons done by substitution of the deficient substance. In particular lifelong application of vitamin B₁₂ is necessary when malabsorption has been defined. Treatment of gall stones and kidney stones is not different from that in patients without IBD. Prophylaxis can be done using dietary measures in particular for kidney stones (excess calcium, low oxalic acid).

In summary extraintestinal symptoms in IBD should be better acknowledged since they can lead to early diagnosis when occurring prior to the intestinal symptoms and also can actually compromise life quality, which can be improved when they are diagnosed and treated.
Innate immunity - Microbial products
Good bugs and clinical use of probiotics in the treatment of IBD

F. Shanahan
Cork University Hospital, Department of Medicine, Cork, Ireland

Abstract not received by the copy deadline.
We have adapted to live in homeostasis with a very rich and complex microbial ecosystem in the distal intestine by developing redundant protective mechanisms. Innate and acquired immune responses to exclude microbial organisms, adjuncts and antigens; clear and degrade invading agents; and downregulate pathogenic immune responses to commensal bacterial and dietary antigens and adjuvants. Chronic intestinal inflammation can develop when these interacting protective mechanisms are defective. Observations in rodent models and human IBD demonstrate that a variety of genetic defects in mucosal barrier function, bacterial clearance and immunoregulation can lead to chronic T cell-mediated intestinal inflammation. We propose that components of the normal intestinal microflora induce protective responses in normal hosts, but dysregulated detrimental immune responses lead to chronic intestinal inflammation in genetically susceptible hosts. Results in gnotobiotic rodents show that colonization of germ-free hosts with common representative Gram negative or Gram positive enteric bacteria transiently upregulate NFκB in colonic epithelial cells through TLR 4 and TLR 2, respectively. However, by 7 days post colonization, epithelial innate immune responses are downregulated by paracrine or autocrine responses that include induction of TGFβ, IL-10 and PPARγ in lamina propria cells IL-10-/- mice monoassociated with E. faecalis have sustained NFκB activation, absent PPARγ induction and upregulation of TLR 2 mRNA in their epithelial cells. Cecal bacterial lysates and LPS stimulate IL-10 production in wild type antigen presenting cells (APC), with abundant production of IL-10 by dendritic cells and B cells. This bacterial-induced IL-10 in APC is a key determinant of tolerance vs. TH1 immune responses. Both pathogenic and protective immune responses are selectively induced by different commensal bacteria and bacterial components, some of which are mediated by TLR and possibly NOD 2/CARD 15. We have demonstrated that intestinal inflammation in animal models is specific for bacterial species and host genetic background. Even in a single inbred mouse strain, different bacterial species can induce different phenotypes of disease. We suggest that the relative balance of detrimental vs. protective (probiotic) bacterial species influence mucosal immune responses and may help determine chronic inflammation vs. homeostasis. This balance can be therapeutically manipulated by antibiotics, probiotics and diet (prebiotics).
Defensins and other antibacterial mediators

Charles L. Bevins M.D., Ph.D.
Department of Microbiology and Immunology, School of Medicine, University of California, Davis CA, USA

Intestinal epithelial cells are located at an important interface for host-microbe interaction, and much current investigation has focused on immune responses of this mucosa. The discovery of antimicrobial peptides has increased our understanding of the breadth of mechanisms contributing to mammalian host defense responses.

We, and others, have identified epithelial cells as one source of antimicrobial peptides that contributes to innate immunity in the intestine and other organ systems. In the small intestine, Paneth cells express large amounts of antimicrobial peptides, including lysozyme, sPLA2 and defensins. These peptides are secreted apically into the intestinal lumen. Recent evidence indicates that proteolytic processing of the defensin precursor molecules (prodefensins) is key to their regulated expression. Paneth cell antimicrobials likely contribute to host defense of the small intestine through selective antibiotic activity, influencing the composition and limiting the numbers of transient and resident lumenal microbes in the lumen. Recent data support that Paneth cell defensins may contribute substantially to host defense against \textit{S. typhimurium} and other pathogens from food and water born sources.

In addition to antimicrobial peptides, others have discovered that Paneth cells also express NOD2, a receptor linked to the recognition of the bacterial cell wall component muramyl dipeptide. Mutations in NOD2 are linked to genetic susceptibility in approximately one third of individuals with Crohn’s disease. Current hypotheses on the pathogenesis of Crohn’s disease hold that luminal bacteria may initiate and propagate intestinal inflammation in genetically susceptible individuals. Recent investigation has found that Crohn’s patients with disease in the ileum have a decreased expression of Paneth cell defensins. The decreased defensin expression was not observed in either colonic Crohn’s disease or ulcerative colitis. Given the tissue localization of Paneth cells in the small intestine and the functional impact of defensins on luminal bacteria, a decrease in Paneth cell defensins may provide a further understanding of mechanisms underlying ileal Crohn’s disease.
Functional consequences of mutations in pattern recognition receptors

Gabriel Nuñez
Department of Pathology and Comprehensive Cancer Center, University of Michigan, Ann Arbor, USA

The elimination of infectious agents by the host plays a critical role in the survival of metazoans. The sensing of microbial agents by the host is mediated by the recognition of pathogen-associated molecular patterns (PAMPs) that are highly conserved structures expressed uniquely by microbes of the same class. PAMPs are recognized by specific host pattern-recognition receptors (PRRs). These PRRs include Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-containing proteins (Nods). Mammalian Nod proteins are structurally related to cytosolic plant disease resistance (R) proteins and comprised of three distinct functional domains: an amino-terminal effector domain involved in signaling, a centrally located regulatory NOD domain, and carboxyl-terminal LRRs that serve as a ligand-recognition domain.

There is now conclusive evidence that two Nod proteins, namely NOD1 and NOD2, function as PRRs to induce signaling pathways upon recognition of bacterial PAMPs in the cytosol. NOD1 and NOD2 recognize conserved but distinct structural motifs in bacterial peptidoglycan through their LRRs and induce the activation of NF-kappaB. NOD1 and NOD2 mediate NF-kappaB activation through RICK/RIP2, a serine/threonine kinase that interacts with the IkappaB kinase (IKK) complex. Importantly, mutations in three Nod family members have been implicated in inflammatory diseases and/or immunodeficiency. Two types of disease-causing variants, loss-of-function and constitutive active mutations, have been observed. In some cases, these mutations involve homologous residues in different Nod proteins, suggesting a common molecular mechanism of disease. Loss-of function mutations in CIITA cause type II bare lymphocyte syndrome, a hereditary disorder characterized by severe immunodeficiency. Mutations in the NOD2 gene (also called CARD15) have been implicated in two inflammatory diseases. Three major NOD2 mutations, R702W, G908R and L1007fsinsC, as well as multiple rare variants, have been found to be associated with susceptibility to Crohn's disease (CD). These CD-associated NOD2 variants are deficient in their ability to sense PGN and/or synthetic MDP. A role for defective NOD2 signaling in CD is supported by analysis of peripheral blood mononuclear cells (PBMC) from individuals homozygous and heterozygous for the common NOD2 mutations. Notably, NOD2 mutations are associated with early-onset and ileal involvement of disease but much less with disease in which the ileum is spared, suggesting that CD can be subclassified according to NOD2 genotype.

The precise mechanism by which loss-of function NOD2 mutations increase the susceptibility to CD is presently not understood. Certain individuals homozygous for the main NOD2 mutations are healthy, suggesting that in addition to NOD2, other genetic and/or environmental factors contribute to the development of CD. The observation that the response to bacterial components is impaired in CD-associated
NOD2 variants suggests that a deficit in sensing certain bacteria by NOD2 may trigger secondary intestinal inflammation, perhaps in response to uncleared bacteria or bacterial products in the lamina propria. Intriguingly, NOD2 is expressed in Paneth cells and might regulate the anti-bacterial function of Paneth cells, an activity that may be impaired in CD patients. Consistent with this notion, recent studies have revealed a link between NOD2 and expression of alpha-defensins, a class of anti-microbial peptides produced by Paneth cells. Another possibility to explain the role of NOD2 in this inflammatory disease is that NOD2 plays a negative regulatory role in the immune response to bacteria and NOD2 deficiency could lead to inappropriate activation of T-cells in intestinal tissues.

In addition to CD, several missense mutations of NOD2, R334Q, R334W, and L469F, have been associated with the development of Blau syndrome (BS), a monogenic, dominantly inherited disease characterized by early-onset granulomatous arthritis, uveitis, and skin rashes. The disease-causing mutations involve residues located in the NOD domain of NOD2 and exhibit constitutive NF-kappaB activity compared to wild-type NOD2. Although both CD and BS are characterized by the presence of granulomatous inflammation, they are clearly different diseases with distinct clinical presentation and mode of inheritance. Unlike CD, there is no evidence that bacteria play any role in the pathogenesis of BS.

Three autosomal-dominant diseases, familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS), and chronic infantile neurological cutaneous and articular syndrome (CINCA), are associated with missense mutations in the CIAS1 gene which encodes Cryopyrin. The Cryopyrin-associated diseases represent closely related autoinflammatory syndromes characterized by recurrent episodes of fever, skin rashes, and tissue inflammation. Disease-causing missense mutations involve residues extending throughout the entire NOD domain of Cryopyrin. Several mutations, including R260W and D303N, are located in the ABC region of the NOD domain, potentially affecting the active conformation of Cryopyrin. R260W, another Cryopyrin mutation associated with FCAS and MWS, corresponds to the R334W NOD2 gain-of-function mutation found in BS. Notably, some disease-causing mutations corresponds to identical or closely located residues in the switching region of NOD2 whose mutation results in constitutive activation. Functional studies have revealed that the disease-associated Cryopyrin mutants R260W, D303N, and E637G exhibit a gain-of-function phenotype with enhanced ASC recruitment and IL-1beta secretion when compared to the wild-type protein. Consistent with these studies it was found that unstimulated blood cells from patients with MWS and CINCA secreted constitutively elevated levels of pro-inflammatory cytokines including IL-1beta. An important role for dysregulated IL-1beta in disease pathogenesis is supported by clinical improvement after treatment of MWS patients with IL-1 receptor antagonist. The molecular mechanism that confers constitutive activation to disease-associated Cryopyrin and NOD2 mutations is presently under investigation.
Postinfectious IBS – Role of inflammation and gut flora

Prof. Robin C. Spiller
The Wolfson Digestive Diseases Centre, University Hospital, Nottingham, NG7 2UH, Great Britain

While most cases of IBS begin insidiously, around 1 in 10 describe an acute onset after a bout of gastroenteritis. Risk factors for developing postinfective irritable bowel syndrome (PI-IBS) in order of decreasing importance are: severity of initial illness, bacterial toxigenicity, female sex, hypercondriasis, anxiety and depression and age < 65 years. PI-IBS patients also show increased mucosal T lymphocytes, increased IL-1β mRNA and increased 5HT-containing enteroendocrine cells. When both psychological and histological variables have been examined and multivariate analysis has been performed these appear to be independent but equally important risk factors. Gut permeability is also increased and this may persist for many years. Genetic differences in the immune modulating genes such as IL-10 promoter may predispose to delayed resolution of inflammation and IBS. Typical symptoms are abdominal pain, frequent, urgent loose stools associated with bloating and the passage of mucus per rectum. The prognosis is variable with only 40% recovering by 6 years. Short duration anti-inflammatory treatment with steroids has not proven effective but whether safer anti-inflammatory therapies such as probiotics might be effective remains to be evaluated.

References:


Gut-liver interactions - Basic and clinical aspects
The liver and its contribution to induction of oral tolerance

Percy A. Knolle, M.D.
Institute for Molecular Medicine and Experimental Immunology, Bonn, Germany

Orally ingested antigens typically are not considered by the immune system as a target for immunity although these antigens are clearly recognized and presented on MHC I and MHC II molecules to CD8 and CD4 T cells, respectively. Regulatory CD4 T cells, generated by tolerogenic dendritic cells in mesenteric lymph nodes, which drain antigen and antigen-loaded dendritic cells from the gut, are most important to induction of immune tolerance towards orally applied antigens. However, oral antigens are not strictly compartmentalized to the gut and the gut-associated-lymphatic-tissue but distribute systemically via the blood stream within minutes to hours after antigen ingestion. Here, we demonstrate that scavenger liver sinusoidal endothelial cells (LSEC) are instrumental in uptake and cross-presentation of oral antigens to CD8 T cells in the liver. LSEC bear most efficient mechanisms to cross-present even minute concentrations of antigen on MHC I molecules. Denaturation of antigen, e.g. by exposure to low pH, leads to improvement of cross-presentation by two log steps in LSEC but not in dendritic cells. Gastrointestinal passage of antigen thus favors MHC-restricted presentation rather in the hepatic context than in secondary lymphatic tissue. As a consequence of cross-presenting oral antigens, LSEC render CD8 T cells tolerant. Naïve OVA-specific CD8 T cells adoptively transferred into RAG2 knockout animals loose their ability to produce effector cytokines and fail to show antigen-specific cytotoxicity in vivo, if these animals received LSEC from mice previously fed OVA. Moreover, using a new transgenic mouse line expressing the MHC I molecule H-2Kb exclusively on endothelial cells (tie-2-H-2Kb), we provide evidence that CD8 T cells are tolerised towards oral antigens. Collectively, our data demonstrate a role of the liver, in particular scavenger LSEC, to contain systemic CD8 driven immune responses towards blood-borne antigens that are derived from the gastrointestinal tract.
The molecular regulation of lymphocyte homing between the liver and gut

David H. Adams, Bertus Eksteen, Allister Grant, Alice Miles and Trish Lalor
Liver Research Group, MRC Centre for Immune Regulation, Institute of Biomedical Research, University of Birmingham B 152TT, Great Britain

Primary sclerosing cholangitis is characterized by progressive bile duct destruction developing as an extra-intestinal complication of inflammatory bowel disease. However, the liver and bowel inflammation are rarely concomitant and PSC can develop in patients whose colons have been previously removed. Thus any model to explain the development of hepatic complications in IBD needs to take into account the fact that colectomy does not alter the severity or course of PSC and that liver disease frequently runs an independent course from inflammation in the bowel. Naïve lymphocytes primed to gut-derived antigens acquire at least two crucial molecules that allow specific gut-homing; \( \alpha_4 \beta_7 \) integrin and the chemokine receptor CCR9 which are expressed by more than 90% lymphocytes in the small bowel. MAdCAM-1, the ligand for \( \alpha_4 \beta_7 \), is expressed widely in mucosal vessels and related lymphoid tissue and is the predominant adhesion molecule in the intestinal lamina propria. Up-regulation of MAdCAM-1 during gut inflammation leads to the sustained recruitment of circulating \( \alpha_4 \beta_7^+ \) lymphocytes and the establishment of chronic bowel inflammation. Homing to the small bowel is enhanced by the restricted expression of the chemokine CCL25 by crypt and glandular epithelium and small bowel venular endothelium. Ligation of CCR9 by CCL25 triggers conformational activation of \( \alpha_4 \beta_7 \) binding to MAdCAM-1. Lymphocytes that home to the colon show limited expression of CCR9 but instead express the chemokine receptor CCR10 which allows them to respond to the mucosal associated chemokine CCL28 on colonic mucosal vessels and epithelial cells. In order to explain the associated between PSC and IBD we hypothesised that effector T cells generated in the organised lymphoid tissue of the gut during active IBD persist as long-lived memory cells that may be recruited to the liver by aberrantly expressed gut-specific adhesion molecules and chemokines thereby mediating extra-intestinal inflammation in the absence of active IBD. In support of this we have shown that liver-infiltrating lymphocytes in PSC include CCR9 and CCR10+ mucosal T-cells recruited to the liver in response to aberrant expression of MADCAM-1 on liver endothelium and ectopic expression of the gut-specific chemokines CCL25 and CCL28. CCL25 activation of CCR9 results in the \( \alpha_4 \beta_7 \)-dependent binding of mucosal lymphocytes to MAdCAM-1 on hepatic endothelium and CCL28 activation of CCR10 \( \alpha_4 \beta_1 \) binding to VCAM-1 on biliary epithelium. This is the first demonstration in humans that T cells activated in the gut can be recruited to an extra-intestinal site of disease and provides a paradigm to explain the pathogenesis of extra-intestinal complications of IBD.
Current concepts in celiac disease

Detlef Schuppan, M.D., Ph.D.
Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Celiac disease (cd) is an inflammatory small intestinal disorder which can lead to global malabsorption. It is triggered by the gluten proteins of wheat, barley and rye. Classical cd as characterized by diarrhoea and malabsorption is relatively rare (prevalence 1:2,000 – 1:4,000 in the West). However, by using the highly predictive autoantibody screening (IgA anti-endomysial or anti-tissue transglutaminase, tTG, autoantibodies) and confirmation by duodenal biopsy, much higher numbers of patients with atypical, silent or asymptomatic cd are found (prevalence 1:80 – 1:200 in Europe, the USA, North Africa and the near and middle East). If and how far these patients, when remaining undetected and thus untreated, are at a high risk of developing other autoimmune diseases, osteoporosis, or cancer, remains to be shown. Virtually all cd patients share HLA-DQ2 and/or -DQ8 on chromosome 6p21 as common genetic background. Genes other than HLA-DQ2 and -DQ8 contribute to cd, notably clusters on chromosome 5q31-33 and 19, and perhaps 2q33. HLA-DQ2/DQ8 bind gluten peptides and present them to intestinal T cells, resulting in T cell activation which drives destruction of the resorptive villi. Almost all patients with cd develop immunoglobulin A autoantibodies to the enzyme tTG which is expressed by many cell types and associates with the extracellular matrix (endomysium or reticulin). tTG targets certain glutamine residues in some extracellular and intracellular proteins tethering them to a lysine residue of a second protein which results in crosslinking of protein chains. With their high glutamine content of up to 50% gluten proteins are ideal substrates for tTG. Importantly, the enzyme can deamidate glutamine to glutamic acid residues which creates negatively charged gluten peptides that bind more strongly to HLA-DQ2 and -DQ8 and hence potentiate T cell stimulation. There are at least 50 tTG target sites and thus immunogenic gluten epitopes in most wheat variants, and similar numbers in barley or rye. The manifestation and severity of cd is determined by the amount of gluten ingested, by the gene dose of HLA-DQ2/DQ8, and likely by additional microbial and chemical triggers. Since patients are under significant dietary and thus social constraints, nondietary therapies for cd are desirable. The development of such therapies will profit from development of a suitable mouse model of cd, such as transgenes with stable expression of DQ2 (DQ8), and other predisposing genes such as DR3. Treatment options are 1. proteases that degrade immunodominant gluten peptides in the proximal gut (prolyl endopeptidase), 2. inhibitors of tTG, 3. DQ2 (DQ8) binding and T cell receptor blocking compounds, and 4. immunomodulators that induce anergy or (oral) tolerance instead of an inflammatory T cell response to gluten.
Hepatobiliary manifestations occur frequently in inflammatory bowel disease (IBD) of both Crohn’s disease (CD) and ulcerative colitis (UC) types. Primary sclerosing cholangitis (PSC) displays prevalences as high as 8% in UC and 3% in CD. Idiopathic pericholangitis, often labelled “early PSC,” involving the small bile ducts reaches a prevalence as high as 30% in IBD. Less common associations such as autoimmune hepatitis and primary biliary cirrhosis are probably coincidental, and fatty liver generally reflects disease severity and nutritional status. Gallbladder stones are frequent in IBD and are emerging as the first hepatobiliary manifestation to be molecularly understood. Prevalence is two to four times that expected for age and gender and, as with nephrolithiasis, typifies ileal CD but not UC patients. Historically, gallbladder bile in CD patients with ileal disease and/or resection was shown to be supersaturated with cholesterol [cholesterol saturation index (CSI) > 1], and compositional analysis revealed that the gallstones were cholesterol in type (Cohen et al, 1971; Dowling et al, 1972). More recent studies (Lapidus & Einarsson 1991, 1998) have suggested that gallbladder bile in CD patients exhibits biliary CSIs ≤ 1 and that the stones are black pigment (polymerized calcium bilirubinate) in composition (Magnuson et al, 1989; Brink et al, 1999). The earlier cholesterol stone concept was supported by studies with controlled interruption of the enterohepatic circulation in rhesus monkeys (Small et al, 1970) where mild bile acid losses increased relative biliary cholesterol saturation (CSI > 1). However, experiments in ileectomized prairie dogs bolstered the contrary concept, since they revealed an increased risk for pigment gallbladder stones (Pitt et al, 1984). Moreover, partial ileal bypass for surgical control of hypercholesterolemia (Buchwald et al, 1999) resulted in formation of gallstones that were also pigment in type. We had discovered earlier (Brink et al, 1996) that in ileectomized rats there are two- to three-fold increases in biliary secretion of bilirubin conjugates (hyperbilirubinemia), findings suggestive of enterohepatic cycling (EHC) of unconjugated bilirubin because of bile salt malabsorption. To explain these apparently divergent results with respect to CSI and bile salt loss, the studies of Färkkilä (1988), mostly carried out in CD patients, are pivotal. He showed that, after ileal resection, the CSI of bile depended critically upon the extent of resection and ensuing fecal bile acid loss. Relative cholesterol composition of bile increased with small bile acid losses, whereas with major losses CSI decreased because of shunting of hepatic cholesterol into upregulated de novo bile acid synthesis. In summary, there are increased risks for two types of cholelithiasis in CD patients, which can now be explained as follows: Prior to the onset of IBD, the prevalence of cholesterol stones and pigment stones should be the same as in a healthy cohort population (e.g., ~ 9:1). Once ileal CD begins, then modest bile salt malabsorption leads to CSI > 1, placing the patient at additional risk for cholesterol gallstones. However, with massive bile salt losses from more severe ileal CD or resection, the CSI decreases because of upregulated de novo bile acid synthesis. Moreover, the excess spillage of bile salts into the colon leads to solubilisation and enterohepatic cycling of unconjugated bilirubin, the latter being
produced by bacterial $\beta$-glucuronidase deconjugation of bilirubin conjugates. Hyperbilirubinemia is the principal risk factor for black pigment gallstone formation, particularly when biliary CSI < 1 (Cahalane et al, 1988). It seems auspicious, therefore, to revisit the other major hepatobiliary manifestations in IBD with new insights, especially as to whether PSC and pericholangitis of both UC and CD have a pathogenetic origin in a hepatotoxin that is normally excreted via the gut.
Primary biliary cirrhosis (PBC), cholestasis and autoimmune liver disease
Primary biliary cirrhosis remains an enigmatic autoimmune disease characterized by antimitochondrial antibodies and immune mediated destruction of bile ducts. There have been significant advances in our understanding of the immune response in patients with PBC and there are a number of “signatures” that relate to both etiology as well as effector mechanisms. Firstly, the vast majority of patients with PBC produce antimitochondrial antibodies; approximately 50% of AMA negative patients by immunofluorescence can be found to have autoantibodies when recombinant autoantigens are used as substrates. Secondly, the presence of antimitochondrial antibodies, when recombinant antigens are used, are virtually diagnostic of PBC; if AMAs are discovered incidentally in an asymptomatic patient, the data strongly imply that patients will develop PBC in 5-10 years. Thirdly, patients with PBC have autoreactive CD4 T cells that react within a similar epitope as autoantibodies. In fact, the liver specific autoreactive CD4 T cell precursor frequency is 100-150 fold higher than it is in blood. Fourth, using tetramer technology, patients with PBC have a higher number of CD8 specific autoreactive T cells in liver than in blood and the level correlates with disease stage. Fifth, the autoepitopes recognized by autoantibodies as well as autoreactive T cells encompass the inner lipoyl domain and patients can be shown to have specific antibodies to lipoic acid. Such data implies that post-translational modification of lipoic acid may lead to neoantigen formation and the generation of an immune response in genetically susceptible hosts. Sixth, the mitochondrial autoantigens are highly conserved, suggesting that molecular mimicry plays a role in disease pathogenesis. In fact, we identified an organism called Novosphinobium aromaticivorans that has four lipoyl domains and the highest degree of homology with the human enzyme. Patient sera react to this organism that titers up to 1:1,000,000, the levels of which are often 1,000-fold higher than against E. Coli. Novosphingobium aromaticivorans is also unique because it metabolizes aromatic hydrocarbons and also converts environmental estrogens to active beta estradiol. Seventh, patients with PBC react to chemical xenobiotic modifications of the inner lipoyl domain, often with higher titer than to the native antigen. These data, from points six and seven, suggest that the etiology may have more than one common thread and involve not only exposure to bacteria, but also chemical modification. Eighth, although other organisms have been suggested, including an MMTV-like retrovirus, such data has not been reproduced and is likely an artifact. Finally, PBC is found in considerably higher frequency within first degree relatives and the concordance of PBC in identical twins is more than 60%. The constellation of these data suggest that PBC is a genetic disease with an environmental component and a long latency period.
Primary biliary cirrhosis: Clinical aspects

Raoul Poupon
Hôpital Saint-Antoine, AP-Hôpitaux de Paris, Inserm U680, Faculté de Médecine Pierre et Marie Curie, Paris, France

Nowadays, the majority of individuals given a diagnosis of primary biliary cirrhosis (PBC) have an early-stage disease. When treated with ursodeoxycholate (UDCA, ≥ 13 mg/kg/day) these patients have a normal life expectancy (1). However in a minority of them, PBC remains active and progresses towards cirrhosis and eventually liver failure. It is thus imperative to establish early valid predictors of disease progression with the aim to identify patients who need aggressive adjuvant therapies.

Accordingly, two studies from our group were carried out (1, 2). In both studies, we found that the following variables were associated with both histological progression and incidence of death or liver transplantation: serum bilirubin and albumin levels, serum alkaline phosphatase and alanine aminotransferase activities, interlobular bileduct paucity and lymphocytic piecemeal necrosis. However, multivariate analysis indicates that only the serum bilirubin level (cut-off 1 mg/dl) and lymphocytic piecemeal necrosis grade for a given histological stage predict independently both development of cirrhosis and incidence of liver transplantation. Other centers have reported that the presence of other autoimmune disease or the pattern of antinuclear antibodies and HLA antigens could help to identify patients with a more severe disease (3-6).

Open pilot studies have reported the beneficial effects of several combination therapies in patients with suboptimal response to UDCA. However, available controlled trials failed to demonstrate a beneficial effect of combination therapies in patients with a suboptimal response to UDCA. Nevertheless, importantly the combination of corticosteroids and UDCA has been demonstrated to be superior to UDCA alone in patients of daily practice (7-9). The effect of this therapeutic option has not been assessed in patients with early markers of active disease under UDCA therapy. In this presentation we will report our experience in this setting. It suggests that the combination of UDCA and corticosteroids is mandatory in the subset of patients who present with both the features of PBC and autoimmune hepatitis. Further, another subset of patients with marked portal and periportal inflammation, low serum bilirubin levels and lacking severe ductopenia could benefit from this combination. A third subset of patients characterized by marked ductopenia without extensive fibrosis are resistant to corticosteroids plus UDCA and always needs liver transplantation.
References:


Molecular mechanisms of cholestasis

Michael Trauner, M.D.
Laboratory of Experimental and Molecular Hepatology, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University, Graz, Austria

Cholestasis may result either from a functional defect in bile formation at the level of the hepatocyte or from an impairment in bile secretion and flow at the bile duct level. Hepatobiliary transport systems are responsible for hepatic uptake and excretion of bile salts and other biliary constituents (e.g., bilirubin) into bile. Reduced expression and function of transport systems plays an important role in the pathogenesis of cholestasis. In addition to transporter changes, other mechanisms such as altered cell polarity, disruption of cell-to-cell junctions and cytoskeletal changes may be involved. Transport defects may be hereditary due to genetic defects or acquired as a result of cholestatic injury. Several monogenetic, hereditary cholestatic syndromes now can be attributed to specific mutations of individual hepatobiliary transporter genes. Examples include progressive familial intrahepatic cholestasis (PFIC), benign recurrent intrahepatic cholestasis (BRIC), Dubin-Johnson syndrome and liver involvement in cystic fibrosis. Knockout mice for the canalicular phospholipids flippase Mdr2 (Abcb4) (corresponding to human MDR3 /ABCB4) develop a sclerosing cholangitis as a result of toxic, phospholipid-deficient bile which contains free, non-micellar bile salts. This Mdr2−/− mouse model may have important implications for human diseases resulting from MDR3 defects, possibly including (primary) sclerosing cholangitis. Incomplete or heterozygous transport defects may predispose to acquired cholestatic liver injury (e.g., subtypes of intrahepatic cholestasis of pregnancy, drug-induced cholestasis). Exposure to acquired cholestatic injury (e.g., drugs, hormones, proinflammatory cytokines, biliary obstruction or destruction) can also result in altered expression and function of hepatic uptake and excretory systems, changes which may maintain and contribute to cholestasis and jaundice. Recruitment of alternative efflux pumps and induction of phase I and II detoxifying enzymes may limit hepatic accumulation of potentially toxic biliary constituents in cholestasis by providing alternative metabolic and escape routes. Generally, cholestasis is associated with a disruption of the normal enterohepatic circulation, with an increased cholehepatic shunting (reabsorbing toxic, stagnant bile form the obstructed ducts in post-canalicular forms of cholestasis) and an increased basolateral efflux of biliary compounds from liver followed by their renal elimination. These adaptive transporter changes may be assisted by metabolic changes of phase I and II enzyme systems involved in the detoxification of bile salts and other biliary compounds which make them less toxic and better substrates for alternative elimination pathways.

These molecular changes are mediated by bile salts, proinflammatory cytokines, drugs and hormones at a transcriptional and posttranscriptional level. Liver-enriched transcriptions factors (e.g., hepatocyte nuclear factors) and nuclear (orphan) receptors for bile salts, bilirubin and drugs play a key role in the transcriptional regulation of hepatobiliary transport. In addition to transcriptional mechanisms, posttranscriptional events (affecting mRNA processing and stability) and/or posttranslational changes (such as impaired targeting and sorting, transporter
redistribution, enhanced transporter protein degradation, direct protein modifications (e.g., (de-) phosphorylation, (de-) glycosylation), changes in membrane fluidity or cis-/trans-inhibition of transport systems by cholestatic agents (e.g., drugs) can also play an important role in the pathogenesis of cholestasis.

Alterations of hepatobiliary transporters and enzymes are not only relevant for a better understanding of the pathophysiology of cholestatic liver diseases, but may also represent important targets for pharmacotherapy. Drugs (e.g., ursodeoxycholic acid, rifampicin) used to treat cholestatic liver diseases and pruritus may counteract cholestasis via stimulation of defective transporter expression and function. In addition, therapeutic strategies may be aimed at supporting and stimulating alternative detoxification pathways and elimination routes for bile salts in cholestasis. Several drugs used empirically in the treatment of cholestasis have retrospectively turned out to be nuclear receptor agonists. Recently, more specific agonists have been developed which remain to be tested under experimental and clinical cholestatic conditions.

References:


Autoimmune liver diseases

Christian P. Strassburg
Division for Gastroenterology, Hepatology and Endocrinology (Director: Prof. Dr. M.P. Manns), Hannover Medical University, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

The autoimmune liver diseases are divided into three distinct clinical pictures all of which are characterized by an aetiologically poorly understood loss of tolerance towards the liver by the immune system manifesting in various tissue compartments. In autoimmune hepatitis (AIH), the hepatocytes are immunologically attacked; in primary biliary cirrhosis (PBC), the smallest intrahepatic bile ducts are destroyed, whereas the large intra- and extrahepatic bile ducts are destroyed in primary sclerosing cholangitis (PSC). Mainly females contract PBC and AIH. All three diseases are chronic inflammatory liver diseases and will progress to liver cirrhosis if they remain untreated.

Therefore, therapeutic requirements focus on the consequences and complications of liver cirrhosis and portal hypertension, which are equally present in the three clinical pictures. On the other hand, extrahepatically manifest, immunologically mediated diseases including autoimmune thyropathy, Sicca syndrome (and Sjögren’s syndrome), rheumatoid arthritis and collagenosis, all of which require a more specific therapeutic strategy, are characteristic particularly for PBC and AIH. Finally, these therapeutic considerations are combined with a specific therapy of the respective liver diseases PBS, PSC or AIH, which are distinctly different.

Autoimmune hepatitis

From a serologic view, autoimmune hepatitis can be divided into three subgroups. Patients with AIH type 1 have antinuclear autoantibodies and antibodies against smooth muscle cells (ANA, SMA); AIH type 2 is characterized by liver-kidney microsomal autoantibodies (LKM-1), and antibodies against soluble liver antigen/liver-pancreas antigen (SLA/LP) are detected in AIH type 3. The clinical presentation of the serologically defined subgroups differs: middle-aged patients are more frequently met with AIH type 2 for about 10 years, with a higher risk of progression to cirrhosis and an acute course of the disease, whereas 80% of the patients suffering from AIH type 1 are 16 to 30 years old at diagnosis and this subgroup shows a rather unspectacular course of the disease. However, the serologic subclassification of AIH has no impact on the therapeutic strategy and cannot be used as the basis for differential-diagnostic considerations. Diagnosis of AIH is characterized by exclusion of viral (hepatitis A, B, C, E, CMV, HSC, VZV, EBV), genetic (M. Wilson, haemochromatosis, alpha-1-antitrypsin deficiency) and toxic (drugs, alcohol) causes of a liver disease. Nearly 90% of the patients are female and serum immunoglobulins are elevated. Autoantibody diagnosis points the way for the future. A probability score (“autoimmune hepatitis score”), which has been internationally validated (see Alvarez et al., 1999) can be employed for autoantibody diagnosis in difficult cases.
**Standard therapy of AIH:** Therapy of AIH aims at induction and maintenance of remission. Standard therapy of AIH is indicated when the aminotransferases exceed the 5 to 10-fold value of the upper standard range, when there is histological evidence of multilobular or bridge necrosis or when serious hepatic or extrahepatic symptoms are present (fatigue, upper abdominal pain, associated immune syndromes). Treatment of patients with complete cirrhosis without inflammatory activity, of old patients or of patients with mild inflammation and without signs of fibrosis is not indicated.

Equally effective is monotherapy with prednisone or combination therapy with prednisone and azathioprine. The decision of administering combination therapy depends on the patient profile: older, osteoporotic patients with metabolic syndrome or with psychological instability are candidates for the combination of prednisone with azathioprine, which spares steroids. Prednisone monotherapy is indicated in pregnancy (to avoid suspected teratogenic effects of azathioprine) and in cases of cytopenia and can additionally be used for diagnostic purposes for short-term induction therapies. Both therapy variations should follow strict regimens to avoid unintended recurrence. Prednisone monotherapy starts with oral administration of 60 mg daily, which can be reduced to 20 mg over a period of 4 weeks. This dosage can be further reduced in steps of 2.5 mg per week with checking of the aminotransferases. In combination therapy, 50 mg daily p.o. is combined with prednisone 30 mg daily p.o., which is reduced to 10 mg daily p.o. or less within 4 weeks. Induction of remission is attained within 3 years in 87% of the cases. There is, however a high recurrence rate of 70% within 3 years after discontinuation of immunosuppression, which means that lifelong immunosuppressive therapy is required in most cases. There is a 10-year survival rate of 90% with drug-induced remission. AIH was the first chronic liver disease to show dramatic improvement of prognosis with drug therapy. If remission cannot be attained after 4 years, liver transplantation is a definite therapeutic option.

**The issue of induction of remission:** Induction of remission fails in about 10% of cases. Therefore, alternative immunosuppressive drugs derived from transplantation medicine are applied in these cases. Cyclosporine A combined with prednisone was used in the therapy of children with AIH types 1 and 2 and resulted in histologically established improvement. The treatment was well tolerated. Cyclophosphamide was used in combination with steroids; however, it was associated with serious haematological side effects. In a pilot study, mycophenolate showed normalization of liver values in 5 of 7 patients within 3 months. The transplant immunosuppressive agent tacrolimus (FK506) resulted in improvement of aminotransferase and bilirubin values in a collective of 21 patients. However, these alternative immunosuppressive agents are reserved for studies in hepatologic centres due to their serious side-effect profile.

**The issue of remission maintenance:** Alternative strategies are required for cosmetic reasons, for maintenance of compliance of younger patients and patients with osteoporosis, psychological problems or other factors that have a negative impact on the indication of steroid therapy. It was demonstrated that maintenance of remission (but not induction of remission) could be attained by administration of oral azathioprine (2 mg/kg bodyweight daily), which therefore, represents a steroid-free
alternative. The use of the topical steroid **budesonide** is another therapeutic option. Its advantages as an alternative to prednisone treatment are considered to be an over 90% hepatic first-pass metabolism, high glucocorticoid receptor affinity as well as degradation to inactive metabolites. However, these advantages are limited in cases of portosystemic shunts and advanced cirrhosis. Overall, there is still controversy as to the benefit of budesonide; the possibility of remission induction is being evaluated in current studies.

**Therapy and prophylaxis of osteoporosis:** In the majority of patients with AIH, lifelong therapy is necessary, which means combination treatment of azathioprine and prednisone in most cases. In these patients, prophylaxis involving a combination of **vitamin D** with **calcium** is indicated. Moreover, intermittent **biphosphonate therapy** (e.g., alendronate 10 mg daily p.o. or sequentially etidronate 400 mg over 14 days and calcium 50 mg over 76 days) is recommended.

**Diagnosis of primary biliary cirrhosis**

PBC is diagnosed via serological detection of atimitochondrial antibodies (AMA with PDHE-2 or BCKD-E2 specificity), the cholestatic liver enzyme profile with elevation of gamma glutamyl transferase and alkaline phosphatase, serum immunoglobulin M elevation and by histological detection of inflammatory bile duct involvement in the liver biopsy. Sonographically and in endoscopic retrograde cholangiography (ERC), the bile ducts are not enlarged. Conspicuously, there is, however, a high number of extrahepatic diseases and symptoms of rheumatic disorders, which may precede diagnosis of liver disease and frequently require symptom-oriented treatment of arthralgias and other symptoms (therapy using non-steroidal antiphlogistics). These symptoms include rheumatoid arthritis, mixed collagenosis, the CREST syndrome, immune thyropathy, celiac disease, inflammatory bowel disease and lupus erythematoses.

**Standard therapy of primary biliary cirrhosis:** Unlike AIH, PBC does not respond to immunosuppressive treatment. Immunosuppressive agents including prednisone, azathioprine, methotrexate and thalidomide were administered with disappointing results or with a pronounced side-effect profile. The standard treatment consists of oral administration of ursodeoxycholic acid 13-15 mg per day. This therapy has been evaluated in 548 patients during observation periods of up to 4 years. It shows an improvement of biochemical serum parameters including the prognostically highly relevant bilirubin (Mayo prognostic model), improvement of survival and of portal hypertension. Fatigue and osteoporosis respond poorly, however. Treatment with ursodeoxycholic acid is well tolerated and should also be consistently applied in the early stages of the disease.

**Therapy of complications of primary biliary cirrhosis:**

**Pruritus:** Therapy of pruritus is often clinically problematic. In severe cases, pruritus may justify liver transplantation even in the presence of good liver performance. For effective management of pruritus a staged treatment regimen is recommended. First, cholestyramine should be applied in doses of 4 to 16 g p.o. daily. 90% of the patients will experience an improvement of their symptoms. Ursodeoxycholic acid, which is used in PBC, has also an antipruritogenic effect. In the second stage, antihistaminic
agents (e.g., terfenadine 2 x 60 mg p.o. daily) or inducers such as rifampicin (2 x 300 mg p.o. daily) are administered. In a third step, serotonin antagonists, e.g., ondansetron (3 x 4-6 mg p.o. daily) or the orally usable opioid antagonist naltrexone (50 mg p.o. daily) may be administered. In refractory pruritus, therapy may be tried using propofol, metronidazole or plasmapheresis. Finally, liver transplantation remains as the ultima ratio therapy.

**Sicca syndrome:** The Sicca syndrome is symptomatically treated using artificial tears.

**Osteoporosis:** The prevalence of PBC-associated osteoporosis is unknown, but estimated to amount to 30% of (female) patients. It is probably due to a so-called “high turnover” disorder, which is characterized by disturbed osteoblast-mediated bone mineralization. Generally, an active lifestyle as well as a balanced diet rich in minerals and vitamins is recommended. Particularly physical inactivity, which is frequently associated with liver disease, should be avoided. Combined substitution of calcium (1000-1500 g p.o. daily) and 1,25(OH)2 vitamin D3 (500-5000 IU p.o. daily) is recommended. Since absorption of all fat-soluble vitamins including vitamins A, E and K is decreased in cholestatic diseases, substitution of all four vitamins is recommended. Administration of calcitonine (40 U Karil i.m. 3 times per week combined with calcium and vitamin D3) has a beneficial effect. The use of biphosphonates in PBC (e.g., alendronate 10 mg p.o. daily or sequentially etidronate 400 mg over 14 days and calcium 50 mg over 76 days) is still being controversially debated. A therapeutic effect was demonstrated so far for alendronate only.

**Prevention:** Cholestasis-dependent malabsorption of fat-soluble vitamins (vitamins A, D, E, K) should be compensated for by substitution. The cholestyramine dose should be adjusted if necessary or a different anti-pruritus agent used. Checking TSH is recommended in view of the association with immunothyropathy in 15-20% of cases.

**Diagnosis of primary sclerosing cholangitis:**

PSC is characterized by progressive destruction of large intra- and extrahepatic bile ducts and – unlike AIH and PBC – affects mainly males (64%), with a maximum age of 24 to 40 years. Ulcerative colitis is associated with PSC in approximately 75% of cases. PSC is characterized by upper abdominal pain, pruritus, anorexia, and fever, and up to 50% of the patients may be asymptomatic. Diagnosis is based on the typical biochemical profile of cholestasis with elevated serum bilirubin, alkaline phosphatase and gamma glutamyl transferase, the characteristic findings of ERC as well as liver biopsy, which may show a ring fibrosis of bile ducts. Serologically, atypical antineutrophil cytoplasmatic autoantibodies (xANCA) are detected in up to 80% of patients. PSC is significantly associated with cholangiocellular carcinoma (10-20%) and colonic carcinoma (9% with 10 years).

**Standard therapy of primary sclerosing cholangitis:** PSC is a progressive disease and is not curable by means of drug therapies. The therapy of choice in the early and late stages of the disease is ursodeoxycholic acid in dosages higher than those used in PBC (15-20 mg/kg bodyweight p.o. daily). Lower doses (below 10 mg per kg of bodyweight) are ineffective. It was reported in three studies that administration of ursodeoxycholic acid resulted in histological improvement and reduction of the prognostically decisive serum bilirubin. The results of
immunosuppressive therapeutic approaches were disappointing or have not been validated sufficiently yet. If episodes of cholangitis cannot be treated, bilirubin is increasing or portal hypertension progressing, liver transplantation remains as the last therapeutic option.

**Endoscopic therapy:** The decisive factor determining the clinical course of PBC is the formation of bile duct stenoses, which promote the development of septic cholangitis. Endoscopic dilatation and short-time stenting (10F prostheses over 10 days) result in improvement of cholestasis and decreased intervention frequency. The combination of endoscopic intervention with the administration of ursodeoxycholic acid leads to a significant prolongation of the transplant-free period and survival. Ursodeoxycholic acid alone does not attain this effect.

**Adjuvant therapy:** Recurrent episodes of septic cholangitis are in the foreground of PSC and require antibiotic therapy. Otherwise, the symptom-oriented therapeutic approaches to cholestatic liver disease should be applied as described for PBC.

**Therapy of portal hypertension:** Treatment of portal hypertension is indicated for all chronic liver diseases leading to cirrhosis. Provided that the circulation is stable it includes administration of β-blockers for reducing portal venous pressure (propranolol 80-200 mg p.o. daily) In cases of acute bleeding complications vasopressin i.v. as well as endoscopic intervention procedures should be applied in the hospital. Applying a transjugular portosystemic shunt (TIPSS) should be discussed if high-grade encephalopathy can be excluded and if sufficient liver performance is present.
Primary sclerosing cholangitis (PSC), alcoholic steatohepatitis (ASH), non-alcoholic steatohepatitis (NASH)
Basic concepts in PSC

Dr Peter T. Donaldson, Ph.D.
School of Clinical Medical Sciences - Liver, University of Newcastle, Newcastle upon Tyne, Great Britain

The aetiology of PSC remains largely unknown. Despite being considered one of the three common “autoimmune” liver diseases, PSC does not fit the classical definition of an autoimmune disease. The majority of patients are male, do not respond to corticosteroid therapy and organ or tissue specific autoantibodies have not been identified. One observation which does support the concept of autoimmunity is the consistent reports of strong genetic associations with specific HLA haplotypes. Yet even this may be questioned. Strong HLA associations have also been reported in viral liver disease and liver diseases due to adverse drug reactions [1]. Whatever the answer to this debate, it is clear that genetics may be very informative in PSC. Indeed genetics may be at the heart of understanding PSC.

The basic concept to consider is that genetics may be useful in understanding disease pathology and may have applications in disease management. The most important questions, for us to consider in this presentation are: - how does genetics inform both our understanding of disease pathogenesis and how can this information be used in disease management?

Genetically PSC behaves like most “autoimmune diseases.” Thus, genes alone are not sufficient to explain PSC. PSC is a genetically “complex disease” (i.e. it does not display a simple Mendelian pattern of inheritance). Consequently, there is no single PSC gene, rather one or more genes may act (either alone or in concert) to increase (or reduce) the risk of disease or of a particular clinical phenotype. In PSC the most obvious phenotypes (clinical subgroups) relate to the presence or absence of inflammatory bowel disease (IBD), cholangiocarcinoma and the rate of disease progression or fibrosis.

The majority of genetic studies in PSC involve case-control association studies of the major histocompatibility complex (MHC). So far six different HLA haplotypes have been found to be associated with PSC (table 1). There are currently three possibilities to explain these HLA associations:

[1]. That susceptibility maps to the HLA class II region of the MHC. According to this theory HLA class II alleles associated with an increased risk of PSC may have properties that favour binding and presentation of one or more autoantigenic epitopes and thereby increase the risk of disease in susceptible individuals. A number of models have been proposed to explore this hypothesis [2]. Conceptually this proposal would favour PSC being a T-cell mediated autoimmune (or infectious) disease.

[2]. That susceptibility maps to the MHC class III region (between HLA B and HLA DR). One proposal is that the primary susceptibility locus is the MICA (MHC-class I chain-like A) locus, which may be important in regulating NK and NK/T cell
function [3]. This proposal favours the possibility that relative differences in the regulation of innate immunity may provide an immune response profile favourable for the genesis of PSC and this may suggest PSC is an infectious as opposed to an autoimmune disease. Though the distinction may be academic as infectious agents may also trigger latent autoimmunity in a susceptible host.

[3]. That there are multiple susceptibility alleles on each haplotype. Conceptually this may explain some of the phenotypic variation in PSC with different risk haplotypes promoting different immune response profiles each having different downstream consequences. In this regard it is important to note the central role played by NK cells in tumour surveillance and consider the hypothesis that possession of specific MICA alleles may be permissive for tumour development. It is also important to note that there are marked differences in the HLA associations in patients with and without IBD.

Aside from these MHC haplotypes there are many other potential candidate PSC promoting alleles in the human genome. There are currently three major areas of interest. First, many investigators have sought to identify defects in molecular transporters in PSC patients. Most studies have been negative [4]. Though there is some (albeit controversial) suggestion of a relationship between PSC and mutations in the Cystic Fibrosis gene - CFTR. Second, PSC is essentially a fibrogenic disease and inherited variation in genes controlling matrix metabolism have identified some interesting associations, most notably with the matrix metalloproteinase-3 (MMP3) locus. Fibrosis is a common pathway in disease and therefore any genetic association with such genes is unlikely to be disease specific [5]. Third, the majority of PSC patients have IBD, and considerable progress has been made in the search for IBD alleles. It is very likely that one or more of these IBD alleles will be implicated in the pathogenesis of PSC [2].

In terms of the basic concepts in PSC these are very exciting times. Genetic data are beginning to inform both pathogenesis and prognosis in other related diseases (especially in Crohn’s disease). The hope is that this type of information will be applicable to understanding the aetiology of PSC and its many phenotypic characteristics. In diseases like PSC genetics offers one of the best options for understanding disease pathogenesis – but it is not the only option. The advantage of this approach is that whilst pathology progresses, changing with time, the patient’s genes do not change (in this context) offering a timeless window into disease pathology.

Table 1: The principle HLA haplotypes in PSC.2, 3

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>B8-TNFA<em>2-MICA</em>008-DRB3<em>0101-DRB1</em>0301-DQA1<em>0501-DQB1</em>0201</td>
<td>2.69</td>
</tr>
<tr>
<td>DRB3<em>0101-DRB1</em>1301-DQA1<em>0103-DQB1</em>0603</td>
<td>3.8</td>
</tr>
<tr>
<td>DRB5<em>0101-DRB1</em>1501-DQA1<em>0102-DQB1</em>0602</td>
<td>1.52</td>
</tr>
<tr>
<td>DRB4*-DRB1<em>04-DQA1</em>0301-DQB1*0302</td>
<td>0.26</td>
</tr>
<tr>
<td>DRB4*-DRB1<em>0701-DQA1</em>0201-DQB1*0303</td>
<td>0.15</td>
</tr>
<tr>
<td>MICA*002</td>
<td>0.12</td>
</tr>
</tbody>
</table>
References:

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Clinical concepts in PSC

Ulrika Broomé, M.D., Ph.D.
Department of Gastroenterology and Hepatology, Karolinska University Hospital, Karolinska Institute, Huddinge, Sweden

Clinical concepts in PSC can be divided into four different areas. 1. Onset of disease 2. Follow up 3. Timing of liver transplantation and 4. Follow up after liver transplantation. At onset of disease it is of importance to do a proper diagnosis, characterisation of the disease and prognostication. Diagnosis is made from a combination of clinical, biochemical, radiological and histological features. Each diagnostic tool is being hampered by overlap with other liver diseases and secondary causes to sclerosing cholangitis have to be excluded. The development of magnetic resonance tomography (MRC) provides a non-invasive diagnostic tool with no known side effects or complications, which further leads to increased identification of PSC. The extent of the involvement of the biliary tree has to be defined - patients having only small bile duct PSC seems to do better than those having large duct involvement. Do the patient have a dominant stricture or not - if so dilatation with brushings should be considered. Is cirrhosis present and if not, do the patient have signs of portal hypertension anyhow? Almost half of all PSC patients with oesophageal varices do not have liver cirrhosis and endoscopy has to be done on wide indications. Is the patient asymptomatic or symptomatic? Today, almost half of all PSC patients are asymptomatic at time of diagnosis and the prognosis is better for these patients. The symptoms in PSC can be related to chronic cholestasis, end stage disease and portal hypertension and also to associated disorders the most important being ulcerative colitis. Moreover, patients may suffer from abdominal pain fever and fatigue. All patients with PSC should undergo colonoscopy in order to search for inflammatory bowel disease. The disease process in PSC is highly variable in the individual patient and also between patients and it can be difficult to do a proper prognostication. The most feared complication in PSC is the development of cholangiocarcinoma, which affects 10-15% of all patients. None of the prognostic models are able predict cholangiocarcinoma development.

There is at present no treatment that can halt the disease process and therapy shall therefore focus on treatment of complication and symptoms of the disease in order to improve patient’s life quality. The only curative treatment remains to be liver transplantation. Liver transplantation in PSC has excellent survival rates and PSC has become one of the most important indications. Optimal timing is, however, of great importance for outcome. Data has, however, emerged that the long term results after liver transplantation is worse for PSC than for primary biliary cirrhosis. This is partly explained by recurrence of PSC after liver transplantation, which seems to have impact on long-term outcome.

Patients with PSC are at increased risk of developing cancer at the sites exposed to chronic inflammation such as the biliary tree, the colon and the pancreas.

Treatment with UDCA decreases the risk of developing colorectal dysplasia/cancer in PSC patients with UC, which certainly is a glimmer of hope for these patients. The role of UDCA for cancer prevention of the liver has to be evaluated in the future.
Alcoholic liver disease

Prof. C.P. Day
University of Newcastle, The Medical School Center for Liver Research, Newcastle upon Tyne, Great Britain

Alcoholic liver disease (ALD) remains the most common liver disease presenting to liver units across Europe and is currently the commonest indication for transplantation. Steatosis, the first stage of ALD, develops in the majority of heavy drinkers and is considered to be an inevitable biochemical consequence of excessive alcohol consumption. It is reversible with abstention from alcohol, however, it may play a role in the progression to more advanced forms of disease - alcoholic hepatitis and cirrhosis. Studies performed in animal models of steatosis have suggested that fat increases the sensitivity of the liver to a variety of injurious mechanisms thought to play a role in alcohol-related hepatocyte injury. Accordingly, weight loss and the use of insulin sensitising agents, recently shown to be of value in non-alcoholic fatty liver disease, may be worth examining in patients with alcohol-related fatty liver. Alcoholic hepatitis occurs in only 10-40% of heavy drinkers. Mechanisms of necroinflammation include endotoxin-induced release of pro-inflammatory cytokines, oxidative stress arising from alcohol metabolism and immunological mediated injury. The short-term prognosis of patients with acute alcoholic hepatitis can be predicted from Maddrey's discriminant function (DF) (which is based on bilirubin and prothrombin time prolongation) and the Glasgow Alcoholic Hepatitis Score (GAHS), which also includes white cell count, age and renal function, and is more specific for mortality than Maddrey's DF. Corticosteroids reduce mortality in patients who are not bleeding or septic, with an early (7 days) fall in bilirubin associated with a 6 month survival of >90%. There has also been a positive trial reported using the anti-TNFα agent pentoxifylline and encouraging results have been reported from studies with anti-TNFα antibodies and aggressive enteral nutrition. Alcoholic cirrhosis develops in less than 10% of heavy drinkers. Prognosis depends on subsequent alcohol intake and the presence of decompensation at presentation. No specific treatments have been shown to improve prognosis, although the anti-oxidant agent, S-adenosylmethionine (SAMe) shows some promise. For patients who remain decompensated despite a period of abstinence, the outcome of liver transplantation is at least as good as for other indications. Patients with Childs Grade C (and possibly Grade B) cirrhosis have better long-term survival with transplantation than without. The incidence of true recidivism appears to be low, in the order of 10%, however, as expected, recent studies with long follow-up, have shown significant morbidity and mortality in patients returning to heavy drinking following transplantation.
Role of the immune system in the pathogenesis of non-alcoholic steatohepatitis

Anna Mae Diehl
Department of Medicine, Duke University, Durham, North Carolina, USA

Studies of animals and humans with obesity-related fatty liver disease have taught us much about the cellular mechanisms that mediate this common pathology. This presentation will summarize pertinent information about obesity-related dysfunction that occurs in hepatic immune cells and demonstrate the effects this exerts on other types of liver cells. Recent studies suggest that obesity-related fatty liver disease is intimately related to the insulin resistance (or metabolic) syndrome. The metabolic syndrome is a constellation of disorders, including obesity, type 2 diabetes, dyslipidemia and hypertension, that result from abnormal production of hormones and cytokines that regulate inflammatory responses. Individuals with the metabolic syndrome over-produce pro-inflammatory factors relative to anti-inflammatory factors. For example, they exhibit increased levels of TNF alpha but relatively low levels of its antagonist, adiponectin. The pathophysiologic relevance of an increased TNF:adiponectin ratio for obesity-related fatty liver disease is proven by animal studies. Treatments that inhibit TNF activity (e.g., neutralizing TNF antibodies) or that increase adiponectin (e.g., supplemental adiponectin) both improve liver injury in obese mice. Despite strong evidence that tissue damage in the metabolic syndrome results from a chronic inflammatory state, the immunologic deficits that sustain inflammation in various target tissues, including the liver, remain poorly understood. The liver is a major component of the innate immune system, housing huge numbers of tissue macrophages and specialized lymphocytes, such as natural killer T (NKT) cells. Studies of mice that become obese and develop the metabolic syndrome and fatty liver disease due to genetic deficiency of leptin reveals that these mice have an abnormal innate immune system. Their livers are selectively depleted of NKT cells due to increased rates of NKT cell apoptosis. This is accompanied by Th-1 polarization of other cytokine-producing liver mononuclear cells, leading to excessive production of pro-inflammatory cytokines, such as TNF and IFN-γ, but decreased production of anti-inflammatory Th-2 cytokines, such as IL-4. Treating ob/ob mice with norepinephrine (to correct their inherent deficiency of this neurohumoral factor) reduces NKT cell apoptosis, restores hepatic NKT cell populations, inhibits the hepatic production of Th-1 cytokines and significantly enhances Th-2 cytokine expression by liver mononuclear cells. The resultant Th-2 polarization of hepatic cytokine production decreases TNF hepatotoxicity, as evidenced by normalization of serum ALT levels. However, the combination of norepinephrine and excessive Th-2 cytokines induces the proliferation of hepatic stellate cells, up-regulates hepatic expression of the profibrogenic cytokine, transforming growth factor (TGF)-β, induces collagen gene expression and leads to peri-sinusoidal fibrosis. These findings underscore the importance of neurohumoral and immunologic mediators in regulating the hepatic response to injury.
Liver failure and transplantation
Acute liver failure - Mechanisms of liver cell destruction

C. Trautwein, C. Klein, T. Lüdde, U. Assmus, M.P. Manns
Abteilung Gastroenterologie, Hepatologie und Endokrinologie, Medizinische Hochschule Hannover, Germany

Like no other organ, the liver has the capability to repair organ loss via regeneration. Besides surgical resection of liver cell mass there are various pathophysiological situations that result in liver cell destruction. Examples in humans include viral hepatitis, autoimmune or fatty liver hepatitis.

Various processes control liver cell destruction and regeneration of the liver; cytokines and growth factors play an important role. There is much evidence that they are substantially involved in the control of liver injury and regeneration.

During the past few years our group has focused on two important cytokines – tumour necrosis factor alpha (TNF) and interleukin-6 (IL-6). We were mainly interested in questions concerning liver cell destruction as well as regeneration. In humans, the concentrations of both cytokines were primarily analyzed in patients with acute liver failure and chronic liver diseases. Our studies demonstrated a correlation between the severity of liver injury and the concentration of both cytokines in the livers and sera of the patients.

Animal models were established to enhance our understanding of the physiological role of both cytokines for regeneration and destruction of the liver. For this purpose we manipulated selectively the signal cascades for TNF and IL-6 in hepatocytes. This approach enables the specific analysis of the relevance of the respective signal cascade in hepatocytes in various animal models as well as the targeted characterization of the role of IL-6 or TNF.

Two animal models are given special emphasis in our current studies, concanavaline A-induced liver failure and liver injury caused by ischemia/reperfusion (I/R). In the Con A model we were able to clarify the mechanism of IL-6-dependent protection and to demonstrate novel approaches to therapy.

In the ischemia/reperfusion model we showed that the TNF-dependent NF-kB activation plays a central role: Liver injury was inhibited via genetic modulation of the activity of the IKK complex. Besides genetic manipulation, pharmacological blocking of components of the IKK complex contributed to the inhibition of liver injury. Therefore, these findings represent a promising approach for therapeutic blocking of the I/R injury of the liver.

In summary, targeted manipulation of individual signal pathways in hepatocytes makes possible the molecular characterization of liver cell destruction and the establishment of novel therapeutic strategies.
Clinical management of acute liver failure

Roger Williams
Institute of Hepatology, University College London, London, Great Britain

The three important components of the management schedule are:

1) Identification of causes requiring specific treatment
These include lymphoma, the Budd-Chiari Syndrome, ischaemic hepatic necrosis from left heart failure, or fulminating septicaemia and the rare Wilson’s Disease. Reactivation of HBV in chronic carriers - the commonest cause of ALF in the Far East and seen in the West following chemotherapy treatment, will also respond to specific anti-viral therapy if started early.

2) Early monitoring and optimal intensive care for multi-organ involvement
On this depends the chances of spontaneous recovery and of transplantation being effected. Most series showing a sizable percentage of cases in whom the progression in deterioration of multi-organ failure including cerebral edema cannot be controlled. In a recent one from the UK, 50% survived with medical treatment, 27% died (74% from multi-organ failure, 22% from intracranial hypertension) and 23% underwent transplantation.*

The occurrence of infection, consequent on depression of immune responses, is thought to be an important factor in the progression of encephalopathy and cerebral edema. Controlling infection along with maintenance of the circulation and support of the organs by enhancing tissue perfusion and oxygenation through the administration of NAC remains the cornerstone of supportive therapy, although the benefit of the latter has been questioned. The value of proven hypothermia in lowering intracranial pressure has yet to be proven and although ICP monitoring by bolt insertion may enable more aggressive management although the most recent studies show no survival advantage.

3) Assessment of need for transplantation
Validation of the King’s or Clichy Criteria confirms strong positive predictive values, but a significant percentage (50-60%) of those not fulfilling criteria also progress. One year survival results are 10-20% lower than for elective transplants. Priority allocation systems for Cadaver organs are in place in many countries but in those that do not, the experience to-date with Living Donor Liver Transplantation is encouraging. The MARS liver support device which has been shown to have beneficial corrective effects on the disturbed pathophysiology of ALF may be used to enhance spontaneous recovery and as a bridge to transplant though not yet proven by clinical trial.

*Bernal W, Wendon J. Intracranial hypertension in acute liver failure; prevalence and risk factors for development. Hepatology, Vol. 40, No. 4, Suppl. 1, 2004 A
Current status of liver and bowel transplantation

Prof. Dr. R. Margreiter
Department of General- and Transplant Surgery, University Hospital Innsbruck, Innsbruck, Austria, E-mail: raimund.margreiter@uibk.ac.at

Not only has become transplantation of the liver the therapy of choice for patients suffering from end-stage liver disease, acute liver failure and unresectable liver tumors under certain circumstances, but also intestinal transplantation has become a routine procedure for the treatment of intestinal failure or short bowel syndrome at experienced centers. According to the European Liver Transplant Registry until the end of 2003 a total of 57,665 liver transplants were performed in 51,580 patients in Europe. Approximately the same number was performed in the United States. After an exponential increase in the late 80ies and 90ies the number of liver transplants is plateauing at about 4,800 cases per year due to a lack of suitable organs. In order to overcome organ shortage alternatives to whole organ cadaveric liver transplantation such as split liver transplantation, domino liver transplantation or living related liver transplantation are being used increasingly. They now account for 17 % of all procedures. The most frequent indication for transplantation are cirrhosis of the liver (58 %) followed by primary liver tumors (12 %), cholestatic liver disease (11 %), acute liver failure (9 %) and metabolic diseases (6 %). For patients transplanted after 1988 patient survival is 80 % at 1- and 69 % at 5-years, graft survival 73 % at 1- and 62 % at 5-years. Patients undergoing transplantation for chronic liver disease have a 84 % survival chance at 1-year in comparison to 76 % for those transplanted for a malignant tumor and 66 % for patients with acute liver failure. One-year survival for patients with a single viral infection is 89 %. Over 90 % of patients undergoing a liver transplantation for cancer suffer from hepatocellular carcinoma and only a minor proportion from cholangiocellular carcinoma or bile duct carcinoma. Of the alternative techniques 40 % were split liver transplants, 39 % living donor liver transplants, 13 % reduced size transplants and 8 % domino transplants. Results with these techniques are comparable to whole organ transplantation with 77 % 1-year graft survival after living related and domino transplantation and 65 % and 63 % after split and reduced size transplantation.

The small bowel can either be transplanted alone, together with the liver or as a component of a multivisceral transplant. Primary diseases leading to transplantation are different in pediatric and adult patients. Whereas in children gastroschisis, volvolus, necrotizing enterocolitis, pseudoobstruction and intestinal artresia where the most common indications according to the Intestinal Transplant Registry, intestinal failure in adults was mainly due to ischemia, Crohn's disease and trauma. As of May 31st, 2003 a total of 989 intestinal transplant were performed in 923 patients worldwide at 61 centers. Of these 433(44 %) where isolated transplants, 386 (39 %) combined bowel liver transplants and 170 (17 %) multivisceral transplants. We have performed at our center at total of 24 intestinal transplants of which 15 were isolated bowel transplants and 9 multivisceral transplants. Taking all patients reported to the registry together 1-year graft survival is around 55 % irrespective of the type of transplant. Considering only patients that were transplanted after the year 2000 a graft survival at 1-year for recipients of an intestinal transplant alone was 70 % and patient survival around 80 %.
Anti-cytokine therapy from the bench to the bedside

Charles A. Dinarello
University Colorado Health Sciences Center, Denver, CO, USA

Introduction. Cytokines are regulators of host responses to infection, immune responses, inflammation and trauma. In addition, the same cytokines participate in the healing process. In general, the pro-inflammatory cytokines are not expressed in health. However, cytokines evolved to assist the host in defending itself against infection; once the infection is eliminated, cytokine gene expression returns to being repressed in the nucleosome. In autoimmune diseases, however, cytokine expression appears to continuously and unrelentingly expressed. As a result, tissue damage takes place.

Initial trials blocking cytokines. Attention has focused on blocking cytokines, which are harmful to the host, particularly during overwhelming infection. Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are proinflammatory cytokines and when administered to humans, produce fever, inflammation, tissue destruction and in some cases shock and death. Reducing the biological activities of IL-1 and TNF is accomplished by several different but highly specific strategies; these are neutralizing antibodies, soluble receptors, receptor antagonists and inhibitors of proteases that convert inactive precursors to active, mature molecules. Agents such as TNF neutralizing antibodies, soluble TNF receptors and IL-1 receptor antagonist have been infused into over 13,000 patients in double-blind, placebo controlled trials.

Although there has been a highly consistent small (2-3%) increase in 28 day survival with anti-cytokine therapy, the effect has not been statistically significant. Certain subgroups, such as patients with disseminated intravascular coagulation, show remarkable improvement with anti-TNF therapy. A large meta-analysis of anti-cytokine therapy in sepsis concluded that the benefit was small because the sample size of each trial is small. Ideally, anti-cytokine therapy should be able to "rescue" the patient who continues to deteriorate in the face of considerable support efforts. Unfortunately, it remains difficult to identify those patients who benefit from anti-cytokine therapies in septic shock.

Anti-cytokines in autoimmune disease. In contrast, in autoimmune disease, decreased death is not an end-point to establish efficacy. Blocking IL-1 or TNF has been highly successful in patients with rheumatoid arthritis, psoriasis, inflammatory bowel disease or graft versus host disease but distinctly not in humans with sepsis. Anti-cytokine based therapies have entered clinical medicine. Over 500,000 patients are being treated with agents that reduce the activities of pro-inflammatory cytokines such as IL-1, IL-12, IL-15, IL-18 and TNF. The advantage of anti-cytokine therapies is the high degree of specificity and lack of organ toxicity; the disadvantage is the possible impairment of host defense mechanism. However, physicians understand
the risk-benefit ratio of decreased host defense from patients receiving corticosteroids. Unlike corticosteroids, anti-cytokine therapies have no endocrinologic, metabolic or central nervous system complications. In diseases such as rheumatoid arthritis, psoriasis, cancer and inflammatory bowel disease, there can be a remarkable cessation of the tissue remodeling. This represents a major advance in treating autoimmune diseases. In patients with severe psoriasis, anti-TNFα treatment reverses the disfiguration of the skin without the need for systemic and global immunosuppression. It is also likely that blocking IFNγ will become a therapy for patients with systemic lupus erythematosus.

The clinical spectrum of reducing interleukin-1 activities. Knowing what diseases respond best to a reduction in a specific biological pathway are not always apparent. In the case of reducing IL-1 activities, much is still open to exploration. Presently, anakinra, the recombinant form of the naturally occurring interleukin-1 receptor antagonist (IL-1Ra), has been approved for treating the signs, symptoms and structural damage in patients with moderate to severe rheumatoid arthritis. Over 100,000 patients have been treated with anakinra, some for as long as five years, and many continue to have benefit. But compared to agents that neutralize TNF, clinical responses to anakinra require several weeks or even months of daily treatment. Anakinra binds to the IL-1 receptor type I as a pure receptor antagonist preventing bona fide IL-1 from binding to and activating a cell. Therefore, following a subcutaneous injection of anakinra, blocking IL-1 receptors in the inflamed synovial space is the therapeutic objective in rheumatoid arthritis and for a receptor antagonist, sustaining sufficient receptor blockade is concentration and time dependent. In contrast, direct intra-articular injection of anakinra into osteoarthritic joints provides some patients with pain relief lasting several weeks (1).

There are, however, several systemic multisystem syndromes, which respond to anakinra within hours or days revealing a fundamental role for IL-1 in inflammation. These syndromes are characterized by recurrent fevers, neutrophilic leukocytosis, thrombocytosis, elevated serum amyloid A and C-reactive protein associated with rashes, diffuse and/or frank deforming arthritis. Hearing loss, developmental delay and low grade aseptic meningitis can also be observed in childhood. The symptoms are triggered by mild stresses such as exposure to cold or routine upper respiratory tract viral infections. Anakinra rapidly and dramatically arrests each of the multisystem manifestations of these syndromes, commonly within hours or a few days. Upon cessation of anakinra therapy, clinical signs and symptoms as well as biochemical and hematological abnormalities rebound within days.

These syndromes often occur in patients with single point mutations in a gene called chronic inflammatory autoimmune syndrome-1 (CIAS1) but now termed NALP-3 where the particular protein affected by the mutation is located. The mutations result in single amino acid changes in one of the proteins controlling the activity of the intracellular proteolytic enzyme called caspase-1 (formerly the IL-1β converting enzyme). This enzyme converts the inactive IL-1β precursor molecule into active IL-1β. Active IL-1β is then released from the cell by a tightly controlled secretory process (2). Indeed, monocytes from patients with a mutation release greater amounts of IL-1 than monocytes from subjects without a mutation (3). However, there
are patients with near identical syndromes who lack this particular mutation but experience the same dramatic resolution of disease activity within 24 hours of the first injection of anakinra (4-6). The mutation is also absent in patients with refractory adult onset Still’s disease, where a rapid resolution of the disease activity is observed within hours or days of treatment with anakinra (7-10). Anakinra is now the treatment of choice for patients with steroid refractory adult onset Still’s disease, mutations in NALP-3 and Schnitzler’s syndrome (Jos van der Meer, MD, personal communication). Systemic juvenile rheumatoid arthritis is likely to be another disease best treated with anakinra (11). Familial Mediterranean Fever, a classic disease of recurrent attacks of acute serosal inflammation, is also due to a genetic defect in IL-1β regulation (12). In patients not responding to colchicine, anakinra, rapidly arrests the attacks. The most unexpected response to anakinra has been reported in an inherited disease due to a mutation in the regulation of TNF, not a mutation in IL-1 regulation. These patients experience recurrent fevers and systemic inflammation due to over activity of TNF but respond to anakinra (13).

References:

List of Speakers, Moderators and Scientific Organizers

Prof. Dr. D.H. Adams
Queen Elizabeth Hospital
Institute of Clinical Sciences
Liver Research Laboratories
Birmingham, B15 2TH
Great Britain

M.C. Carey, M.D.
Professor of Medicine
Brigham and Women's Hospital
GI Division
75 Francis Street
Boston, MA 02115-6195
USA

Dr. V. Annese
Ospedale I.R.C.C.S.
"Casa Sollievo Sofferenza"
Divisione di Gastroenterologia ed Endoscopia
I-71013 San Giovanni Rotondo
Italy

Prof. Dr. J.-F. Colombel
Hôpital Claude Huriez
CHRU Lille
Gastroenterology & Hepatology
1, place de Verdun
F-59037 Lille
France

C.L. Bevins, M.D.
Professor of Medicine
University of California
School of Medicine
Med. Microbiology & Immunology
One Shields Ave., Tupper Hall
Davis, CA 95616
USA

Dr. C.P. Day
University of Newcastle
The Medical School
Center for Liver Research
Framlington Place
Newcastle upon Tyne, NE2 4HH
Great Britain

R.S. Blumberg, M.D.
Associate Professor of Medicine
Brigham and Women's Hospital
GI Division
75 Francis Street
Boston, MA 02115-6195
USA

Prof. Dr. Dr. H. Denk
Medizinische Universität Graz
Pathologisches Institut
Auenbruggerplatz 25
A-8036 Graz
Austria

Prof. Dr. U. Broomé
Karolinska Institute
Huddinge University Hospital
Department of Gastroenterology and Hepatology
Hälsovägen
S-141 86 Huddinge
Sweden

Dr. G. D'Haens
Univ. Ziekenhuis Gasthuisberg
Department of Gastroenterology
Herenstraat 49
B-3000 Leuven
Belgium
A.M. Diehl, M.D.
Professor of Medicine
Duke University Medical Center
Gastroenterology Division
Box 3256, Snyderman/GSRB-1
595 LaSalle Street
Durham, NC 27710
USA

M.E. Gershwin, M.D.
Professor of Medicine
University of California
School of Medicine
Rheumatology & Allergy
One Shields Ave., Tupper Hall
Davis, CA 95616
USA

C.A. Dinarello, M.D.
Professor of Medicine
University of Colorado
Health Sciences Center
B 168
4200 East 9th Avenue
Denver, CO 80262
USA

Prof. Dr. I.W. Graziadei
Universitätsklinik Innsbruck
Klinische Abteilung für gastroenterologie und Hepatologie
Anichstr. 35
A-6020 Innsbruck
Austria

Prof. Dr. P.T. Donaldson
University of Newcastle
The Medical School
Centre for Liver Research
Framlington Place
Newcastle upon Tyne, NE2 4HH
Great Britain

Dr. A. Kaser
Universitätsklinik Innsbruck
Klinische Abteilung für gastroenterologie und Hepatologie
Anichstr. 35
A-6020 Innsbruck
Austria

Prof. Dr. P. Knoflach
Krankenhaus der
Barmherzigen Schwestern
I. Interne Abteilung
Grieskirchnerstr. 42
A-4600 Thalheim/Wels
Austria

Prof. Dr. B.G. Feagan
University of Western Ontario
Robarts Research Institute
LCTR
100 Perth Drive
London, ON N6A 5K8
Canada

Prof. Dr. P.A. Knolle
Universitätsklinikum Bonn
Molekulare Medizin
Sigmund-Freud-Str. 25
D-53127 Bonn
Germany

Prof. Dr. A. Gangl
Universitätskliniken Wien
Gastroenterologie/Hepatologie
Währinger Gürtel 18-20
A-1090 Wien
Austria

Prof. Dr. G.J. Krejs
Medizinische Universität Graz
Auenbruggerplatz 15
A-8036 Graz
Austria

Prof. Dr. U.R. Fölsch
Universitätsklinikum
Schleswig-Holstein, Campus Kiel
Innere Medizin I
Schittenhelmstr. 12
D-24105 Kiel
Germany

Dr. A. Kaser
Prof. Dr. W. Kruis  
Evang. Krankenhaus Kalk  
Innere Medizin  
Buchforststr. 2  
D-51103 Köln  
Germany

Prof. Dr. H. Lochs  
Gastroenterologie/Hepatologie  
Charité Universitätsmedizin  
Campus Charité Mitte  
Schumannstr. 20-21  
D-10117 Berlin  
Germany

Prof. Dr. M.P. Manns  
Gastroenterologie/Hepatologie  
Medizinische Hochschule Hannover  
Carl-Neuberg-Str. 1  
D-30625 Hannover  
Germany

Prof. Dr. R. Margreiter  
Universitätsklinik Innsbruck  
Transplantationschirurgie  
Anichstr. 35  
A-6020 Innsbruck  
Austria

Prof. Dr. F. Mühlbacher  
Universitätskliniken Wien  
Transplantationschirurgie  
Währinger Gürtel 18-20  
A-1090 Wien  
Austria

Prof. Dr. M.F. Neurath  
Klinikum der Universität  
Innere Medizin I  
Langenbeckstr. 1  
D-55131 Mainz  
Germany

G. Nuñez, Ph.D.  
Professor of Pathology  
University of Michigan  
Comprehensive Cancer Center  
Department of Pathology  
1500 East Medical Center Drive  
Ann Arbor, MI 48109-0054  
USA

Prof. Dr. G. Paumgartner  
Innere Medizin II  
Klinikum der Universität  
München - Großhadern  
Marchioninistr. 15  
D-81377 München  
Germany

Prof. Dr. W. Petritsch  
Medizinische Universität Graz  
Auenbruggerplatz 15  
A-8036 Graz  
Austria

D.K. Podolsky, M.D.  
Professor of Medicine  
Massachusetts General Hospital  
School of Medicine  
GI Unit  
55 Fruit Street  
Boston, MA 02114-2696  
USA

Prof. Dr. R. Poupon  
Hôpital Saint-Antoine  
Service d'Hépato-Gastro-Entérologie  
184, rue du Faubourg St. Antoine  
F-75571 Paris  
France

Prof. Dr. D. Rachmilewitz  
Shaare Zedek Medical Center  
Department of Medicine  
P.O. Box 3235  
IL-91 031 Jerusalem  
Israel
Dr. W. Reinisch  
Universitätskliniken Wien  
Gastroenterologie/Hepatologie  
Währinger Gürtel 18-20  
A-1090 Wien  
Austria

Prof. Dr. P. Rutgeerts  
Univ. Ziekenhuis Gasthuisberg  
Department of Gastroenterology  
Heresstraat 49  
B-3000 Leuven  
Belgium

R.B. Sartor, M.D.  
Professor of Medicine  
University of North Carolina  
School of Medicine  
Microbiology & Immunology  
778, Burnett Womack Building  
Chapel Hill, NC 27599-7080  
USA

Prof. Dr. J. Schölmerich  
Klinik für Innere Medizin I  
Klinikum der  
Universität Regensburg  
D-93042 Regensburg  
Germany

Prof. Dr. S. Schreiber  
Innere Medizin I  
Universitätsklinikum  
Schleswig-Holstein, Campus Kiel  
Schittenhelmstr. 12  
D-24105 Kiel  
Germany

D. Schuppan, M.D., Ph.D.  
Professor of Medicine  
Beth Israel Deaconess Medical Center  
Harvard Med. School, Dana 501  
330 Brookline Ave.  
Boston, MA 02215  
USA

Prof. Dr. F. Shanahan  
Cork University Hospital  
Department of Medicine  
Clinical Sciences Building  
Cork  
Ireland

Prof. Dr. R.C. Spiller  
University Hospital  
Queen’s Medical Centre  
Division of Gastroenterology  
C Floor, South Block  
Nottingham, NG7 2UH  
Great Britain

PD Dr. C.P. Strassburg  
Gastroenterologie/Hepatologie  
Medizinische Hochschule Hannover  
Carl-Neuberg-Str. 1  
D-30625 Hannover  
Germany

W. Strober, M.D.  
National Institutes of Health  
NIAID, Bldg. 10, Room 11N 238  
Mucosal Immunity Section  
Bethesda, MD 20892-1890  
USA

PD Dr. J. Tepel  
Allgemein-/Thoraxchirurgie  
Universitätsklinikum  
Schleswig-Holstein, Campus Kiel  
Arnold-Heller-Str. 7  
D-24105 Kiel  
Germany

Prof. Dr. H. Tilg  
Krankenhaus Hall i.T.  
Interne Abt. B  
Milserstr. 10  
A-6060 Hall/Tirol  
Austria
Prof. Dr. M. Trauner
Medizinische Universität Graz
Klinische Abteilung für
Gastroenterologie & Hepatologie
Auenbruggerplatz 15
A-8036 Graz
Austria

Prof. Dr. C. Trautwein
Gastroenterologie/Hepatologie
Medizinische Hochschule Hannover
Carl-Neuberg-Str. 1
D-30625 Hannover
Germany

Prof. Dr. R. Williams
University College Hospital
Medical School
Institute of Hepatology
69-75 Chenies Mews
London, WC1E 6HX
Great Britain

Prof. Dr. M. Zeitz
Innere Medizin I
Charité Universitätsmedizin
Campus Benjamin Franklin (CBF)
Hindenburgdamm 30
D-12203 Berlin
Germany
POSTER ABSTRACTS

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Analysis of an association between NOD2/CARD15 gene variants and response to antibiotics in perianal fistulizing Crohn’s disease

University Clinic of Internal Medicine IV, Division of Gastroenterology and Hepatology,
*Department of Epidemiology, Institute of Tumour Biology, Medical University Vienna, Austria
#Dr. Margarete Fischer-Bosch-Institute of Clin. Pharmacology Stuttgart, Germany

Introduction: NOD2/CARD15 gene variants have been made responsible for susceptibility to Crohn’s disease (CD). NOD2/CARD15 is involved in the immune system’s response to bacteria by recognizing a specific peptidoglycan motif, muramyl dipeptide, and subsequent activating NF-κB. Antibiotics are broadly applied in the treatment of CD owing to their antibacterial activity.

Aims & Methods: This study aimed to explore an association of NOD2/CARD15 gene variants and clinical response of perianal fistulas to antibiotic treatment. 52 patients (f/m: 24/28; median age: 28) with draining perianal fistulas were treated with ciprofloxacin (500-1000 mg/day, n = 41), metronidazole (500-1500 mg/day, n = 3) or both (n = 8) for a median duration of 9 weeks (range 4-28). According to the Fistula Drainage Assessment complete response was defined as fistula closure or the absence of any draining fistulas despite gentle finger compression. Genotyping of the main coding mutations of CARD15/NOD2, denoted SNP 8, 12 and 13, was conducted by TaqMan analysis (Applied Biosystems, Foster City, CA, USA) as previously described by Hampe et al. (Lancet 2002) Laboratory personnel were blinded to case status of the participants.

Results: 13 patients showed complete response to antibiotic therapy, whereas 39 were rated as non- or partial-responders.

Table 1: NOD2/CARD15 Genotype

<table>
<thead>
<tr>
<th></th>
<th>Wildtype, n = 37</th>
<th>Heterozygous/Compound heterozygous, n = 14</th>
<th>Homozygous, n = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-/partial responder</td>
<td>25</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Responder</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Frequency of NOD2/CARD15 Allelic Variants

<table>
<thead>
<tr>
<th></th>
<th>SNP 8</th>
<th>SNP 12</th>
<th>SNP 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>6.7%</td>
<td>2.9%</td>
<td>8.7%</td>
</tr>
<tr>
<td>Non-/partial responder</td>
<td>9%</td>
<td>2.6%</td>
<td>10.3%</td>
</tr>
<tr>
<td>Responder</td>
<td>0%</td>
<td>3.8%</td>
<td>3.8%</td>
</tr>
</tbody>
</table>
At least one mutation in NOD2/CARD15 gene was detectable in 1/13 (7.7%) responder compared to 14/39 (35.9%) non-responder (p = 0.056).

**Conclusion:** These preliminary data suggest a pharmacogenetic significance of NOD2/CARD15 in the treatment of CD for the first time. NOD2/CARD15 wildtype seems to be relevant for efficacy of antibiotic treatment for bacterial flora in perianal fistulas.
Inflammatory bowel diseases - Probiotic treatment

A. Atanassova, M. Georgieva*, I. Kotzev, I. Krasnaliev**
Dept. of Hepatogastroenterology, Dept. of Paediatrics*, Dept. of Pathology**, Medical University, Varna, Bulgaria

**Background:** Several mechanisms have been proposed to account for probiotic action: the antagonist activity against pathogenic bacteria substances such as microbial peptides, stimulation of mucosal defence; blockade of proinflammatory cytokines, enhancement of anti-inflammatory cytokine levels, produce nutrients of special importance such as short-chain fatty acids and vitamins.

**The aim** of the current study was to evaluate the efficacy of treatment with probiotics in patients with inflammatory bowel diseases (IBD).

**Material and methods:** From 01.01.2001 to 01.06.2004 92 IBD - mild and moderate form of ulcerative colitis (UC) and Crohn disease (CD) location in colon were treated with probiotics during the period of relapse and first tree months after clinical remission. 1st group 26 patients with UC and 5 patients with CD received meselazine 3.0 g/daily and Lactobacilli-Streptococcus Cremoris, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacilus casei, Enterococcus faecium, Propioni bacterium, Lactobacillus plantarum, Lactobacillus helveticum, Bifidobacterii-Ferzym-plus® - 6 capsules per day/42 billion/daily for a period of 16 to 20 months. 2nd group of 25 UC patients and 6 CD patients received mesalazine 3.0 g/daily and Saccharomyces boulardii 500 mg/daily (Enterol®) for 16 to 20 weeks. 3rd control group 24 UC patients and 6 CD patients treated by mesalazine 3.0 g/daily without probiotics. All patients were investigated clinically, microbiologically, ultrasonographically, endoscopically and histologically.

**Results:** The two groups with probiotic therapy have shown a significant attenuation of inflammation and stable remission for 9-12 months. In the third group the duration of remission was 6-8 months. These therapeutic schemas were well tolerated, save without adverse reactions.

**Conclusions:** The real efficacy of treatment with probiotics and mesalazine needs further evaluation. This is an attractive new perspective of IBD treatment. It may be associated with other health effects such as immunomodulation.

**Correspondence:**

Dr. Antonia Atanassova
Department of Hepatogastroenterology
University Hospital "St. Marina"
1, Ch. Smirnenski Str.
BG-9000 Varna
Bulgaria
3

Ulcerative colitis associated with adenocarcinoma of the common bile duct

J. Bajor, M. Garamszegi, E. Grexa, B. Anga, K. Szilágyi, T. Beró
Dept. of Internal Medicine and Gastroenterology, Radiology, Pathology and Surgery
Baranya County Hospital, Pécs, Hungary

Bile duct carcinoma is a rare complication of ulcerative colitis. In most of the cases it tends to occur together with primary sclerosing cholangitis predominantly in older males. The authors report a case of a 25 years old women presenting with jaundice, 6 years after the diagnosis of colitis was made. The cause of the extreme extra- and intrahepatic bile duct dilation was revealed by endoscopic retrograde cholangiopancreatography and percutaneous transhepatic cholangiography showing polypoid tumor in the common bile duct. The histological result taken during the surgical exploration proved the diagnosis of adenocarcinoma. Radical pylorus preserving pancreato-duodenectomy was performed. Subsequently adjuvant chemotherapy was instituted according to the PAV protocol. The patient is symptom-free for 4 years, and the restaging examinations are negative. This rare case proves, that a malignant bile duct tumor may develop in a young patient with ulcerative colitis without the presence of primary sclerosing cholangitis. The authors emphasise the connection between ulcerative colitis and bile duct carcinoma and the importance of the close follow-up of patients with ulcerative colitis.
Familial prevalence of the inflammatory bowel diseases and intraabdominal cancers at the patients with ulcerative colitis

Tomasz Banasiewicz, Łukasz Krokowicz, Maciej Borejsza-Wysocki, Anna Jazdzewska, Michal Drews
Department of General, Gastroenterological and Endocrinological Surgery, K. Marcinkowski University of the Medical Sciences, Poznan, Poland

The genetics factors play an important role in development and clinical course of the ulcerative colitis. The observations suggest that genes IBD-1, IBD-2 and IBD-3 on chromosome 6, 12 and 16 are importance in susceptibility of inflammatory bowel diseases. The HLA-DR2 antigen, occur more frequently at the patients with ulcerative colitis, is also more frequently present in autoimmunological diseases, coincidenced with IBD. The aim of the study was to calculate the incidence of inflammatory bowel disorders and intraabdominal neoplasms in relatives of UC patients operated in our clinic.

The aim of the study was to analyze of 96 patients operated due to ulcerative colitis. The patients were divided into two groups depending on the presence (group I, n = 29) or absence (group II, n = 67) of extraintestinal symptoms.

In the group of patients with extracolonic manifestations we observed the higher incidence of the ulcerative colitis and intraabdominal malignant tumors at the family members. Family members associated with group I had an increased incidence of UC when compared to family members associated with group II p < 0.05. The presence of abdominal cancer, as indicated by family history during the medical interview, was also higher in family members of group I p < 0.005.

We observed an increased incidence of UC and abdominal cancers in families of patients that have undergone a surgery due to UC, compared with a healthy population. The positive familial history of patients with occurring extracolonic changes especially draws to attention.
The cytokine modifications in ulcerative colitis with bone complications

S. Bezna, Marinela Bezna, T. Ciurea, C. Vere, Daniela Neagoe, M. Ciurea
University of Medicine and Pharmacy Craiova, Romania

Pathological events in ulcerative colitis (UC) include cellular and humoral mechanisms, frequently modulated by cytokines, among which the tumoral necrosis factor (TNF-α) and interleukin-6 (IL-6) play a prominent part both in inflammation and in the acceleration of bone destruction by activation of osteoclasts.

**Aim:** To determine the variation of TNF-α and IL-6 in patients with UC in relation with the disease activity and the evaluation of osteoporosis by determining the bone mineral density (BMD) and the osteoporotic risk factors.

**Patients and methods:** The study has been made on a group of 24 patients diagnosed with UC, aged between 28 and 56 years, 23 women and 21 men, during a 5 years period.

It has been determined in serum the value of TNF-α and IL-6 by ELISA, in inflammatory activity and in remissions of UC. The osteoporosis has been estimated by osteodensitometry, x-ray, IMR. On explored the gastrointestinal and extraintestinal manifestations at this patients by history, clinical, imagistic (x-ray, endoscopy, ultrasound, biopsy) and biological investigations.

**Results:** TNF-α and IL-6 are multifunctional cytokines with a synergical action. TNF-α and IL-6 presented serum levels 3 to 6 times higher during the active periods of the disease, in relation with the increase of bone demineralisation and the aggression of the inflammatory process. BMD was low, with or without radiological correspondent, both during the active periods of the disease and in remissions, thus demonstrating the high risk of developing osteoporosis at patients suffering of UC. Other risk factors associated with the increase of IL-6 and TNF-α (which mediate the recruitment of osteoclasts and result a generalised bone loss and the decrease of the BMD) included: inflammatory activity, deficient nutrition, smoking, hormonal deficiencies, heredity. The early anti-inflammatory treatment reduced the risk of osteoporosis, except in the case of prolonged corticotherapy.

**Conclusions:**
1. TNF-α and IL-6 are proinflammatory cytokines, involved in the immune and the inflammatory response, which, besides this role, mediate the recruitment of osteoclasts and determine a generalised bone loss in UC.
2. The increase of TNF-α and IL-6 in UC depends on the stage of disease, in excess and with other risk factors been associated with low bone density.
3. Osteoporosis is a complications of UC which can worse the extraintestinal manifestations of disease.
4. The assessment of TNF-α and IL-6, osteoporosis and UC orientate the evolution and the treatment.

**Correspondence:**
Sorin Bezna, Str.N. Balcescu Nr. 12, RO-1100 Craiova, Romania
E-mail: sorinbezna@yahoo.com
Crohn disease with duodenal, enteral and pancolonic extension, complicated by primary sclerosing cholangitis and associated HCV hepatitis

V. Enachescu, M.E. Dinca, I. Opritoiu
III and II Medical Clinic, Clinical Hospital no. 2, University of Medicine and Pharmacy of Craiova, Romania

We present the case of a 38-year old man diagnosed with ileocolonic Crohn disease 19 years ago and with primary sclerosing cholangitis and HCV hepatitis (HAI = 7, fibrosis = 0) 7 years ago, when he received combined treatment with interferon and ribavirin for 5 months. In 2003 his evolution was complicated with diarrhea, epigastric pain and weight loss. On physical examination the patient was malnourished (BMI 16.6), pale, jaundiced, tenderness was noted on palpation of his epigastrum. Laboratory findings: anemia, inflammation markers, increase in serum aminotransferases, cholestasis, hypoalbuminemia, hyposideremia. Upper digestive endoscopy and colonoscopy revealed duodenal and pancolonic Crohn disease. US: tortuous and dilated biliary ducts, enlarged lymph nodes in hepatic hilum. MRI cholangiography: second stage sclerosing cholangitis. Hepatic biopsy: chronic hepatitis (HAI 10, fibrosis 1), second stage sclerosing cholangitis, second stage steatosis. An infliximab application was made with subsequent remission of diarrhea. After discharge the patient remained on azathioprine and UDCA treatment.

After 1 month the patient was readmitted because of melena, epigastric pain, malaise. Upper digestive endoscopy: duodenal ulcerations. After a new infliximab application the patient became febrile and although no infection site was detected, blood cultures were positive for E. coli (bacterial translocation?). Simultaneously, pyoderma gangrenosum and right eye episcleritis occurred. The clinical course was slowly favourable while receiving metil-prednisolone, total parenteral nutrition, large spectrum antibiotics and antifungal drugs.

Correspondence:

Viorela Enachescu
Stirbei Voda street, nr. 16
RO-200374 Craiova
Departament of Dolj
Romania
Phone: ++0040251533516
E-mail: vemd1@yahoo.com, vemd1@umfcv.ro
The prevalence of primary sclerosing cholangitis and antineutrophil cytoplasmic antibodies in patients with inflammatory bowel disease

Amelia Genunche-Dumitrescu, Simona Popa, P. Ciurea, P. Mitrut, Daniela Badea
University of Medicine and Pharmacy, Craiova, Romania

Background: Primary sclerosing cholangitis (PSC) is a chronic, obliterating inflammation of intra- and extrahepatic bile ducts, commonly associated with inflammatory bowel disease (IBD).

The aim of this study was to determine the prevalence of PSC and to investigate the prevalence of antibodies to neutrophil cytoplasmic antigens (ANCA) in our patients with IBD.

Materials and methods: We studied 12 patients with IBD and PSC. The diagnosis of IBD is done according to clinical, endoscopic, radiologic, histochemical and microbiological criteria. PSC was diagnosed in all patients with the use of endoscopic retrograde cholangiopancreatography (ERCP).

Results: PSC was diagnosed in 8 patients with ulcerative colitis UC (6 female, 2 male) and 4 Crohn's disease patients (2 female, 2 male). The average age at the onset of the UC was 22.8 years and 28.5 years Crohn's disease. Two patients diagnosis of PSC preceded the diagnosis of IBD, six had simultaneous diagnosis and four patients PSC was diagnosed after the onset of IBD.
ANCA were found by indirect immunofluorescence (IF) in 8 of 12 patients with IBD and PSC, (6 patients with UC and 2 patients with Crohn's disease). Perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) have been found consistently in patients with UC (5 of 8 patients) and in a much smaller percentage in patients with Crohn's disease (1 of 4 patients).

Conclusions: PSC commonly associated with IBD, especially UC. The prevalence of ANCA is high in patients with UC.
The serum levels of TNF-alpha, IL-1beta, sIL-2R, IL-6, IL-8 and IL-10 in patients with active ulcerative colitis

Vedat Göral, Tahir Celenk, Abdurahman Kaplan
Dicle University School of Medicine Department of Gastroenterology and Biochemistry
Diyarbakir, Turkey

AIM: The etiology and pathogenesis of inflammatory bowel disease (IBD) remains an area under intense investigation. In ulcerative colitis, a T(H)2 T-cell response appears to be the pathological process responsible for the inflammatory disease. To determine the tumor necrosis factor alpha (TNF-alpha), IL-1beta, sIL-2R, IL-6, IL-8 and soluble IL-2 receptor (sIL-2r) in 25 patients with active ulcerative colitis and 20 healthy controls.

METHODS: Twenty patients (9 women, 11 men, mean age 36.2 years) with ulcerative colitis and twenty healthy controls (11 women, 9 men, mean age 27 years) were studied. All cytokines were measured by ELISA method.

RESULTS: TNF-alpha and sIL-2R levels were significantly higher in ulcerative colitis than in healthy controls (TNF-alpha 19.3 pg/ml (4-60.7 pg/ml) vs 9.7 pg/ml (5-20.1 pg/ml) p < 0.05; sIL-2R 1545 U/ml (474-6305 U/ml) vs 537.5 pg/ml (306-931 pg/ml), p < 0.05). IL-1 beta level was not high and there was no relationship between ulcerative colitis and controls. IL-6, IL-8 and IL-10 levels were slightly higher in ulcerative colitis than controls. CRP levels was high around 90% in patients with active ulcerative colitis. No correlation of TNF-alpha, IL-6, sIL-2R were found to disease activity, disease location and medication.

CONCLUSION: Our results show that IL-2R and TNF-alpha are major and key cytokines in patients with active ulcerative colitis in our region. This data demonstrates that the role of cytokines in the pathogenesis of ulcerative colitis.
Antioxidant defence in ulcerative colitis with extraintestinal manifestations

Gotia Laura S.¹ Gotia Smaranda Rodica¹, Susan Lelia²
¹Department of Physiology, ²The IV Department of Internal Medicine, University of Medicine and Pharmacy "V. Babes", Timisoara, Romania

The aim of our study was to investigate antioxidant defence in ulcerative colitis (UC) patients with ulcerative lesions in oral cavity. In UC patients extraintestinal manifestations were found: stomatitis, aphthae, ulcerative lesions, on the oral mucosa.

Reduced glutathione (GSH) is the body's key antioxidant, which works to neutralize oxygen free radicals and thereby prevent or diminish "oxidative stress".

Blood and salivary GSH were determined by spectrophotometric method with dithiobisnitro-benzoic acid. The results showed that in mild form of UC blood GSH was about 439 ± 13.15 micromoles/l but in severe UC, GSH levels was decreased to 224.04 ± 38.55 micromoles/l. Low GSH levels in these patients decrease the ability to inactivate toxic free radicals and oxidative stress could be produced.

Leukocyte number from saliva (760 ± 41.8/microliter), the viability of salivary cells (76 ± 4.17%), were decreased and the number of the epithelial cells was increased (1340 ± 89.4/microliter). The increase of epithelial cells desquamation, the decrease of salivary leukocytes phagocytosis were associated with low levels of salivary glutathione (6.06 ± 2.47 micromoles/l). These modifications can produce epithelial barrier dysfunction, the decrease of local antioxidant defence of buccal mucosa and can explain the presence of ulcerative lesions in oral cavity, in UC patients.

Correspondence:

Dr. Gotia Laura S
Department of Physiology
University of Medicine and Pharmacy "V. Babes"
Timisoara,
2 Eftimie Murgu street, 300041
RO-1900 Timisoara
Romania
E-mail: lauragotia@yahoo.com
Carcinoma arising in IBD is associated with decrease in expression of \( p27^{kip1} \) and increase of \( p53 \) protein

Katarzyna Guzinska-Ustymowicz, Justyna Sieczka, Jolanta Czyzewska, Andrzej Kemona
Department of General Pathomorphology, Medical University of Bialystok, Poland

INTRODUCTION: Patients with ulcerative colitis and Crohn's disease have an increased risk of developing intestinal tumours. Loss of the negative cell-cycle regulator, \( p27^{kip1} \), may contribute to oncogenesis and tumour progression. Whereas increased expression of mutated \( p53 \) has been shown to be involved in carcinogenesis.

MATERIALS AND METHODS: In this study, we have examined expression of \( p53 \) and \( p27 \) proteins in ulcerative colitis, Crohn's disease and carcinomas arising in inflammatory bowel disease (IBD). Twenty-six colon carcinomas (11 from ulcerative colitis, 15 from Crohn's disease) and 55 ulcerative colitis and Crohn's disease were analyzed by immunohistochemistry for cell cycle regulatory proteins (\( p53, p27 \)).

RESULTS: We have observed that carcinoma arising in IBD showed significant decrease in expression of \( p27 \) and increase of \( p53 \) protein. However no differences were observed in the expression of both proteins in cases with ulcerative colitis and Crohn's disease.

Correspondence:

Katarzyna Guzinska-Ustymowicz MD, PhD
ul. Waszyngtona 13
PL-15-889 Bialystok
Poland
E-mail: kguzinska@poczta.onet.pl
"Multi-feature" Crohn disease: Capsule endoscopy of small bowel

M. Kovács, A. Uhlyarik, P. Pák, G. Pák
Vaszary Kolos Hospital, Department of Internal Medicine II, Esztergom, Hungary

Based on literature a 20-30% proportion of Crohn disease can be localised only in the small bowel. Applying traditional radiological methods even well-developed Crohn disease remains hidden. Six suspected and four known Crohn patients were provided capsule endoscopy following negative panendoscopy and colonoscopy carried out due to weight loss, diarrhoea and abdominal pain.

We would like to introduce the macroscopic lesions and the localisation of the small bowel detected during the examinations. General symptoms, laboratory parameters and macroscopic results often showed discrepancies. In four patients so-called skip and early lesions were detected in the duodenum. In all patients examined both the jejunum and the ileum were affected.

Capsule endoscopy provides the opportunity to ascertain the localisation, severity and activity of the disease. The small bowel Crohn disease can be diagnosed at an earlier stage and treated more effectively. This new method is suitable for the measurement of the effectiveness of the therapy.
Investigation of interleukin 10 in Crohn’s disease treated with anti-tumor necrosis factor α antibody

V. Kupcová¹, Z. Zelinková¹, L. Turecký³, M. Príkazská², E. Jahnová²
3rd Department of Internal Medicine, Dérer’s Hospital, Comenius University¹, Slovak Health Care University², Institut of Chemistry, Biochemistry and Clinical Biochemistry, Medical Faculty, Comenius University², Bratislava, Slovakia

Increased serum levels of interleukin 10 (IL-10) were observed in patients with active CD and ulcerative colitis, suggesting that IL-10 acts as a naturally occurring damper in the acute inflammatory process of inflammatory bowel disease. Thus, the administration of anti-TNF antibody might be associated with changes in both, proinflammatory and regulatory parts of the immune system. In an attempt to assess the pattern of immunoregulatory cytokine response in CD patients treated with anti-TNF antibody, serum levels of IL-10 were measured together with clinical and laboratory parameters of disease activity.

Clinical activity (in 14 patients with active, moderate to severe Crohn’s disease), serum IL-10, basic haematological and biochemical parameters (blood count, prothrombin time, renal and hepatic functions) were assessed. All parameters were obtained before treatment in Month 0 (Mo 0) and in Month 1 and 5 (Mo 1, Mo 5) after treatment. Clinical activity was assessed by Crohn’s disease activity index (CDAI). Serum IL-10 was measured by a commercially available kit Quantikine® (R & D Systems). Patients received 5 mg per kg of anti-TNF antibody (infliximab) in intravenous infusion.

Clinical improvement was observed in 12 patients with a decrease in median CDAI from 228 (163-294) before treatment to 98.5 (56-160) in Mo 1; two patients did not respond. According to the clinical response in Mo 1, patients were divided into two groups: Group 1 (7 patients) with a decrease in CDAI of 50% and more; in this group the median of CDAI before treatment was 240 (169-294) diminishing in Mo 1 to 81 (56-125); and the group 2 (7 patients) with a drop of CDAI less than 50%; the median of CDAI in this group was 265 (163-300) before treatment and after 1 month it decreased to 145 (114-294). During the further clinical follow-up, patients in the group 1 remained stable with CDAI of 82 (28-216) in Mo 5, while in the group 2 the clinical activity raised to 203 (108-318) in Mo 5 that did not differ significantly from the clinical activity before treatment.

IL-10 levels before treatment ranged from 3.62 pg/mL to 6.08 pg/mL with a median of 4.44 pg/mL in 13 patients; in one case the IL-10 levels were elevated up to 22.72 pg/mL. During the further follow-up, there was a significant decrease in IL-10 levels in the group 1 (p < 0.05) in Mo 1 and IL-10 levels remained decreased compared to values before treatment in Mo 5. On the other hand, in the group 2 a significant increase in IL-10 levels (p < 0.05) was observed in Mo 1, without significant changes in Mo 5.

We conclude, that the pattern of IL-10 response might play a role in determining the response to anti-TNF therapy.

The work was supported by VEGA grant 1/7531/20.
Erythrocyte-mediated delivery of dexamethasone in steroid-dependent IBD patients: A pilot study


Unità di Gastroenterologia, Ospedale Casa Sollievo della Sofferenza Hospital, I.R.C.C.S., San Giovanni Rotondo, Italy
Istituto di Biochimica, Università di Urbino, Italy
Dipartimento di Medicina Sperimentale e Patologia, Università "La Sapienza", Roma
Istituto Mendel, Casa Sollievo della Sofferenza, Roma
Dipartimento di Medicina Sperimentale, Sezione Biochimica, Università di Genova

Autologous erythrocytes can be used as carriers of drugs, owing to the ability of their membrane to be opened and resealed under appropriate conditions.

Aim: In this pilot study we investigated efficacy and safety of dexamethasone-encapsulated erythrocytes in steroid-dependent IBD patients.

Materials and Methods: Ten patients (5 with ulcerative colitis and 5 with Crohn’s disease) with steroid dependency ranging from 8 to 60 months were studied. Seven of them were in clinical remission, and the remaining 3 had a mild activity. Eight patients were also using azathioprine or 6-MP since at least 6 months (range 6-24 months), while other two patients were intolerant. Fifty ml of blood were drawn from each subject; dexamethasone 21-phosphate (Dex 21-P) was encapsulated into erythrocytes by means of specially designed equipment and drug-loaded erythrocytes were infused into originals donors. The procedure was repeated after 4 and 8 weeks, and patients instructed to withdraw corticosteroids.

Results: A mean dose of 5.5 ± 2.4 mg Dex 21-P was loaded in the erythrocytes at each treatment. Following re-infusion of loaded erythrocytes, plasma dexamethasone (Dex) concentrations was detected as long as 28 days. Steroids were completely withdrawn by the second month. After the third infusion, all patients, including the 3 with mild active disease, were in clinical remission. ESR levels dropped from 47 ± 27 at baseline to 27 ± 16 mm/hr (p<0.02), and CRP levels from 1.6 ± 1.3 mg/dl to 0.6 ± 0.5 mg/dl (p<0.02). After a mean follow-up of 12 ± 3 months, 6 patients relapsed, and the remaining 4 patients remained in remission. Pre-existing steroid-related adverse effects disappeared during the follow-up.

Conclusions: Loading of Dex 21-P in autologous erythrocytes is feasible and safe. The very low dose of Dex released in blood stream was able to maintain patients in clinical remission and allowed steroids withdrawal.
Comparative analysis expression of MMP-9, cathepsin B in inflammatory bowel disease (IBD) and carcinoma arising in inflammatory bowel disease

Katarzyna Guzinska-Ustymowicz¹, Justyna Sieczka¹, Lebel Agnieszka², Andrzej Kemona², Jolanta Czyzewska¹
Department of General Pathomorphology¹, Department of Anatomy², Medical University of Bialystok, Poland

INTRODUCTION: Ulcerative colitis and Crohn's disease are an inflammatory processes of the gastrointestinal tract. Patients with these disease have an increased risk of developing intestinal tumours. The purpose of this study was to examine the cellular location of MMP-9 and cathepsin B in normal, inflammatory and neoplastic cells of the intestine.

MATERIAL AND METHODS: For the study tissue specimens from patients with ulcerative colitis (n = 50), Crohn's disease (n = 14), colon cancer (n = 15) and healthy intestine (n = 6) were examined using immunohistochemical analysis with monoclonal antibodies.

RESULTS: Carcinomas arising in inflammatory bowel disease IBD showed significant increase in expression of cathepsin B and MMP-9 compared to ulcerative colitis and Crohn's disease. Whereas the expression of cathepsin B was much more intensive in cases with Crohn's diseases compare to ulcerative colitis or healthy tissue. These results can suggest that matrix metalloproteinase (MMP-9) and cathepsin B are involved in tissue remodeling, which is one of the important aspects of inflammatory disease and arising carcinoma.

Correspondence:

Katarzyna Guzinska-Ustymowicz MD, PhD
ul. Waszyngtona 13
PL-15-889 Bialystok
Poland
E-mail: kguzinska@poczta.onet.pl
The estimation IL-1 beta, IL-6 and TNF-alfa tissue expression in idiopathic colitis

Ryszard Marciniak, Przemysław Majewski, Jakub Zurawski, Joanna Bierla, Tomasz Banasiewicz, Michał Drews
Department of General, Gastroenterological and Endocrinological Surgery and Department of Clinical Pathomorphology, Poznan University of Medical Sciences, Poland 60-355 Przybyszewskiego 49

The aim of the study was an estimation of the usefulness of the tissue expression of several pro-inflammatory cytokines by immunohistochemistry in differential diagnosis of IBD types.

Analysis was performed on material taken from 11 patients with CD (5 women and 6 men) and 22 with UC (12/10). The patients’ age ranged from 18 to 84 years (mean 53 years) for CD and 14 to 68 years (mean 41 years) for UC. In all patients the colectomy was done and specimens to the histopathological examination were taken. 10 specimens taken from the proximal lines of colon resection due to cancer were used as control. In all cases the routine histopathological examination was done and the frozen section was performed for immunohistochemistry. The immunohistochemical reactions using antibodies against IL-1 beta, IL-6 and TNF-alfa were performed. In IBD cases the extension of the inflammation, its intensity and activity were estimated.

Histopathology allowed to confirm the diagnosis of CD in 11 and UC in 22 cases. In control group there was no inflammatory process in the wall of the colon.

Labeling index (LI) for IL-1 beta was higher in both CD and UC compared to the control group. LI for IL-1 beta was significantly higher in UC than in CD (p < 0.05), and it was correlated with the grade of inflammation.

The estimation of IL-6 expression allowed to observe the highest LI in CD. In UC LI was lower than in CD, but in both types of IBD was significantly higher than in the control group (p < 0.05). In both IBD types IL-6 expression was correlated with the grade of inflammation.

The highest LI for TNF-alfa was observed in CD. In UC LI for TNF-alfa was significantly lower (p < 0.05). Higher values than in the control group were stated only in the cases of the high grade of the inflammatory process. In CD expression of TNF-alfa was correlated with the grade of inflammation.

The results of the study confirm the usefulness of pro-inflammatory cytokine IL-1 beta expression for the estimation of the grade of inflammation in UC. For the estimation of the grade of inflammation in CD cytokines IL-6 and TNF-alfa expressions appear useful.
Clinical significance of anti-neutrophil cytoplasmic antibodies (ANCA) in ulcerative colitis with liver determination

Mitrut Paul, MD, PhD, Genunche Amelia, MD, PhD, Diana Predescu, MD
UMF Craiova, Emergency District Hospital of Craiova, The 2nd Medical Clinic, Romania

Introduction: Anti-neutrophil cytoplasmic antibodies (ANCA) has been detected in ulcerative colitis, Crohn’s disease, autoimmune hepatitis and primary sclerosing cholangitis (PSC), a liver disease that is frequently associated with ulcerative colitis. The clinical significance of ANCA in ulcerative colitis has not been studied in detail.

Aim of study: In this study we analyzed the prevalence of ANCA in ulcerative colitis associated with liver disease.

Patients and Methods: Serum samples were collected from all patients known at the Medical Clinic II, Emergency District Hospital of Craiova with ulcerative colitis (n = 56; 32 male, 24 female; median ages 46 years; range: 22-69 years). The research protocol contained a clinical, biological and a complete imagistic evaluation of the liver and port system. The diagnostic of ulcerative colitis was made in all cases by colonoscopy and biopsy examination. Detection of ANCA by indirect immunofluorescence was performed on ethanol fixed granulocytes.

Results and Discussions: ANCA were present at 62.5% cases with ulcerative colitis. The liver determination was present at 28.5% (16 patients) of cases with the following etiological spectrum: primary sclerosing cholangitis (5.3%), pericholangitis (10.6%), primary biliary cirrhosis (1.7%) and steatofibrosis (10.6%). In this cases ANCA were present at 12 patients (75%) and in forms without liver determination at 26 patients (65%). In primary sclerosing cholangitis (CSP) ANCA was detected in all the cases.

Conclusions: ANCA as detected by indirect immunofluorescence seem to be associated with a severe course of ulcerative colitis. In the forms with hepatic determination, primordially primary sclerosing cholangitis, ANCA is more increased.
Hepatobiliary complications of Crohn's disease

Mitrut Paul, MD, PhD, Genunche Amelia, MD, PhD, Diana Predescu, MD
UMF Craiova, Emergency District Hospital of Craiova, The 2nd Medical Clinic, Romania

**Introduction:** In rare cases the liver and gall bladder are found to be involved in the bowel inflammation in Crohn's disease. Nodular cell proliferation, known as "granulomas", also develops in the liver tissue and the antibodies present in Crohn's disease may also react with the surface of the bile ducts.

**Aim of study:** To investigate the incidence and the profile complications' liver or gall bladder in Crohn's disease.

**Patients and methods:** The study was performed in Second Medical Department of Emergency District Hospital of Craiova on a group of 51 patients known with Crohn's disease. Statistically analysis pointed out the prevalence of male 74.5% (38 patients); average ages 49 years (range 21-69 years). The diagnostic of Crohn's disease was made in all cases by colonoscopy and biopsy examination. The research protocol contained clinical, biological, histological and a complete imagistic evaluation of the liver and port system.

**Results and discussions:** The liver determination was present at 23.5% (12 patients) of cases with the following etiological spectrum: primary sclerosing cholangitis 2 cases, pericholangitis 3 cases, primary biliary cirrhosis 1 case, steatofibrosis 3 cases, colangiocarcinom 1 case and liver cirrhosis 2 cases. In these cases the diagnostic was confirmed by biopsy and histological examination and RMN cholangiography.

**Conclusion:** The liver determination in Crohn's disease is frequently. Specifically was primary sclerosing cholangitis, pericholangitis, steatofibrosis and liver cirrhosis. In patients with Crohn's disease is necessary a complete evaluation (biological and histological) of the liver.
Our experiences with ursodeoxycholic acid in the treatment of ulcerative colitis patients with primary sclerosing cholangitis

Tamás Molnár, Ferenc Nagy
First Department of Medicine, University of Szeged, Hungary

(PSC) is a chronic cholestatic liver disease of unknown aetiology that is generally associated with ulcerative pancolitis. The disease typically progresses slowly, but ultimately, and leads to cirrhosis, liver failure or bile duct cancer. PSC patients with simultaneous ulcerative colitis are also at higher risk for colorectal dysplasia. The aim of our longitudinal study was to determine the long-term outcome of patients with PSC and ulcerative colitis (UC) treated with ursodeoxycholic acid. 20 UC patients with cholangiography or MRCP -proven PSC (14 male, 6 female; mean age 38.2 ± 14.2 years; 16 pancolitis, 4 extended colitis) who were seen at our institution over a 15-year period were followed-up for up to 8 years. All of them had elevated alkaline phosphatase activity and gamma-glutamyl transpeptidase levels at the time of diagnosis. 15 patients presented with symptoms and/or signs of PSC. All patients were treated with ursodeoxycholic acid, alone or in combination with immunosuppressive medications. Positive clinical and/or biochemical response occurred under therapy in 18 patients. 1 of the 2 non-responder patients underwent liver transplantation, while the other died in cholangiocellular carcinoma. During follow-up, no colonic dysplasia or carcinoma was found in the patients treated with ursodeoxycholic acid. Our results suggest a more severe disease course in the patients who does not respond to the ursodeoxycholic acid therapy. Ursodeoxycholic acid may have a protective effect against colonic dysplasia.
The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss

Alexander R. Moschen, Arthur Kaser, Barbara Enrich, Othmar Ludwiczek, Michael Gabriel, Peter Obrist, Anna M. Wolf, and Herbert Tilg
Division of Gastroenterology and Hepatology, Division of Hematology and Oncology, Institute of Pathology, Institute for Medical Chemistry and Biochemistry, Department of General and Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria

Background and aims: A substantial proportion of patients with inflammatory bowel disease (IBD) develops osteopenia and osteoporosis in the course of disease. Recent data from a mouse model of colitis suggest that receptor activated by nuclear factor-kappaB (RANKL)/osteoprotegerin (OPG) system might be responsible for bone loss.

Methods: We investigated the activation state of the RANKL/OPG system and its association with bone loss in human IBD. Plasma levels of OPG and RANKL were correlated with bone mineral density and current IBD therapy. Colonic secretion of OPG and RANKL and cell types responsible for such secretion were determined.

Results: OPG plasma levels were 2.4-fold elevated in Crohn's disease (CD) and 1.9-fold in ulcerative colitis (UC), whereas sRANKL levels were not significantly different in IBD patients compared to healthy controls. High levels of OPG were released from colonic explant cultures (CEC) derived from inflamed IBD specimens, and colonic macrophages and dendritic cells were co-staining for OPG. Soluble RANKL levels from CEC were low both in IBD patients and healthy controls. Interestingly, increased expression of RANKL was mainly confined to cells in the lamina muscularis. A significant negative correlation was found between OPG plasma levels and femoral neck/lumbar spine bone mineral density.

Conclusions: We demonstrate that IBD is associated with alterations in the RANKL/OPG system. Applying results from a murine model of colitis-associated bone loss, the constellation of OPG and sRANKL regulation observed in our study could raise the possibility that RANKL/OPG might contribute to the development of bone loss in IBD.
Extraintestinal conditions associated with inflammatory bowel diseases

O. Petrescu, M. Deac, R. Mihaila
Clinical Hospital, Faculty of Medicine, Sibiu, Romania

Background and aim: The study aims at identifying the types of extraintestinal manifestations, the moment of onset of this associated conditions in relation to the onset of the inflammatory bowel diseases and the way in which it influences the clinical evolution of ulcerative colitis and of Crohn's disease.

Patients and method: We studied 100 patients with ulcerative colitis and 21 with Crohn's disease. We assessed the extraintestinal manifestations, based on clinical data and paraclinical investigations, and they confirm the associated disease diagnosis.

Results: 9 (9%) patients with ulcerative colitis presented articular manifestations: 7 patients had migrant arthritis in one or more articulations; sacroileitis was radiologically confirmed in one patient and, based on confirmation criteria, another one was diagnosed with rheumatoid arthritis. In 3 patients with arthritis and in the patient with rheumatoid arthritis, these conditions were prior to the onset of ulcerative colitis. 2 (2%) patients had skin manifestations, namely erythema nodosum. 4 (4%) female patients with ulcerative colitis were diagnosed with sclerosing cholangitis, which emerged after they had been diagnosed with intestinal disease. In one female patient, the hepatobiliary disease evolved to the stage of secondary biliary cirrhosis. In the group of patients with Crohn's disease, 2 (9.5%) patients had articular manifestations, 1 patient had oligoarthritis, while another one was diagnosed with ankylosing spondilitis with the onset before that of the inflammatory bowel disease.

Conclusions: Articular and hepatobiliary manifestations are the conditions most frequently associated with inflammatory bowel diseases. In certain cases, articular diseases may precede the onset of inflammatory bowel disease. There is no proven connection between the clinical evolution of inflammatory bowel diseases and the existent extraintestinal disease.

Correspondence:

Dr. Ovidiu Petrescu
Spitalul Clinic Judetean Sibiu
Medicala II
Bd. C. Coposu nr. 2-4
RO-24119 Sibiu
Romania
Portal vein thrombosis at a patient with hepatocellular carcinoma and active Crohn's disease

Simona Popa, Amelia Genunche, P. Mitrut
University of Medicine and Pharmacy Craiova, Romania

Portal vein thrombosis must be considered as a clue of existence some prothrombotic disorders associated or not with local factors. The primary hepatocellular carcinoma (HCC) is well known for its predilection to invade the portal vein, with intraluminal tumor thrombosis, while hemostasis measurements complications were performed in patients with Chron's disease (CD).

We present the case of a 55 year-old male patient diagnosed with portal vein thrombosis, type II of cavernous transformation of the portal vein (CPTV), active CD and primary HCC.

The patient presented for diarrhea (6 stools/day), accompanied by weight loss (10 kg in 1 year), abdominal pain. Biological exams indicated anemia (hemoglobin 9 g%), increased erythrocyte sedimentation rate (80/100 mm), increased levels of anticardiolipin antibodies, and a fetoprotein (600 ng/ml). Colonoscopy showed two zones of stenosis, with areas of granular mucosa which alternated with areas of normal mucosa. Biopsies showed chronic inflammation. Upper gastrointestinal endoscopy showed normal gastric mucosa, and normal biopsies. Transabdominal ultrasound showed hepatical tumor, the presence of ascites, and color Doppler ultrasound indicated the main portal vein with a thrombus within the lumen producing collateral circulation - type II of CPTV. In the thrombus no blood flow signals were demonstrated.

In conclusion, the presented case indicated the difficulties of diagnosis encountered at the patient with type II of CPTV witch can be caused by HCC (malignant thrombus when color signals showing arterial flow patterns in the lesion), or by prothrombotic disorders associated with active CD (benign lesion there were no flow signals in the thrombus).
Initial symptoms and liver manifestations in inflammatory bowel disease

S. Sandulescu, Viorela Enachescu, Daniela Tuta, S. Scurtu
Clinical Hospital of Emergency, Department of Surgery, University of Medicine and Pharmacy of Craiova, Romania

Background: Most cases of inflammatory bowel (IBD) disease are accompanied by extraintestinal manifestation which frequently represent initial symptoms of the disease. Many hepatobiliary diseases are seen in IBD and PSC is the most common.

The aim of this study was to discover retrospectively the initial symptoms and hepatobiliary disease in a population with IBD.

Patients and methods: We studied retrospectively 42 patients with inflammatory bowel disease 18 patients with Crohn's disease (12 women and 6 men) and 24 patients with ulcerative colitis (14 women and 10 men).

Results: The period between the initial symptoms and the diagnosis of the disease was 6 months on an average. The most common initial symptoms were abdominal pain, diarrhea, fatigue and weight loss in Crohn's disease, and abdominal pain, bloody diarrhea and anemia for ulcerative colitis. Cholelithiasis, primary sclerosing cholangitis, portal vein thrombosis, fatty liver and hepatic abscess are hepatobiliary disorders that occur in IBD patients.

Conclusion: Inflammatory bowel diseases are less frequent in Romania that in Western Europe. The identification of the initial symptoms and extraintestinal manifestations in inflammatory bowel diseases can help shorten the interval between the initial symptoms and the diagnosis of the disease.

Correspondence:
Sarmis Sandulescu
Dr. Ionescu Sisesti Nicolae street, bl. I82, ap. 19
RO-200303 Craiova
Departement of Dolj
Romania
Telephone: +0040745773593
E-mail: ssarmis@yahoo.com
Therapy resistant ulcerative colitis associated with high MDR1 function

Schäfer E.1-2, Németh B.B.2, Dr. Peták I.2-3, Gyökeres T.1, Hamvas J.1, Schwab R.1-2, Pap Á.1

Department of Gastroenterology, MÁV Hospital1, Budapest, Co-operative Research Centre, Semmelweis University2, 1st Institute of Pathology and Experimental Cancer Research, Semmelweis University3, Hungary

BACKGROUND: Current drugs to treat inflammatory bowel disease (IBD) incl. corticosteroids, azathioprine, cyclosporine are all substrates of ABC-MDR transporters. Expression of these proteins in mucosa infiltrating lymphocytes is not known. Until now, no methods were available to screen patients for individual transporter status, although expression of these proteins can be induced by drug therapy and it may change during ongoing treatment. Testing MDR-transporter function to establish personalized treatment strategies is an increasingly accepted issue by various methods including DNA chip arrays and flow cytometry.

OUR PATIENT: A 70 years old female patient was referred to our unit with therapy resistant ulcerative colitis (UC) involving the sigmoid colon and the rectum. She had no relevant past medical history apart from UC diagnosed 25 years ago with intermittent flare-ups and remissions, multiple boosts of corticosteroids and ongoing ASA therapy. Both clinical symptoms and the endoscopic appearance of the gut mucosa showed sustained inflammatory activity confirmed by histology (UCAI: 9). Functional MDR tests were performed form the gut mucosa and peripheral blood (MDQ test Solvo Biotechnology) showing highly increased MDR1 protein function of mononuclear cells of the gut mucosa and peripheral blood. Increasing the level of methyl-prednisolon resulted in slight improvement in the clinical symptoms, however, still w/o objective endoscopic remission.

CONCLUSION: Controlled clinical studies should address the role of MDR-transporters in therapy-resistant IBD. It is of debate, whether or not transporters, once expressed can be saturated by increasing the dose of drug therapy.
An open-labeled trial of probiotic as a maintenance therapy in patients with antibiotic-dependent pouchitis

Bo Shen¹, Victor W. Fazio², Feza H. Remzi², Aaron Brzezinski¹, Jean-Paul Achkar¹, Conor P. Delaney², Scott A. Strong², Ana E. Bennett³, Marlene L. Bambrick², Kerry Sherman¹, Bret A. Lashner¹
Departments of Gastroenterology/Hepatology¹, Colorectal Surgery², and Anatomic Pathology³, The Cleveland Clinic Foundation, Cleveland, OH, USA

Background and Aims: Management of antibiotic-dependent pouchitis is often challenging. Oral bacteriotherapy with probiotics (such as VSL#3) as maintenance treatment has been shown to be effective in relapsing pouchitis in European trials. However, this agent has not been studied in the US. We conducted an open-labeled trial of VSL#3 in patients with antibiotic-dependent pouchitis.

Methods: Thirty-one patients were enrolled in the study. All patients received 2 weeks of ciprofloxacin 500 mg BID followed by VSL#3 6 grams per day. Baseline Pouchitis Disease Activity Index (PDAI) scores were calculated. Patients' symptoms were reassessed at week 2 when VSL#3 therapy was initiated and at the end of the 8-month trial. Some patients underwent repeat pouch endoscopy at the end of the trial.

Results: All 31 patients responded to the 2-week ciprofloxacin trial with resolution of symptoms and they were subsequently treated with VSL#3. The mean follow-up was 14.5 ± 5.3 months (SD, range: 8-26 months). At the 8-month follow up, only 6 patients were still on VSL#3 therapy (VSL#3 Responders); and the remaining 25 patients discontinued the therapy due to either lack of symptom response or development of adverse effects (VSL#3 Non-responders). The 6 patients in the VSL#3 responder group had a mean treatment period of 14.3 ± 7.2 months (range: 8-26 months) and had repeat outpatient clinical and endoscopic evaluation. At the end of 8-month trial, all 6 patients were virtually asymptomatic with a mean PDAI symptom score of 0.33 ± 0.52. Pouch endoscopy in the 6 patients showed some degree of inflammation with a mean PDAI endoscopy score of 1.83 ± 1.72, which was not statistically different from that at baseline PDAI endoscopy score of 2.83 ± 1.17 (p = 0.27).

Conclusions: This open label trial showed that only a minority of patients with antibiotic-dependent pouchitis were able to maintain symptomatic remission on VSL#3. Given the low therapeutic benefit and the cost of the medication, a controlled trial in the US is required before this therapy is recommended.
Decreased E-cadherin expression in inflamed areas of Crohn's disease

Justyna Sieczka, Katarzyna Guzinska-Ustymowicz, Andrzej Kemona, Jolanta Czyzewska
Department of General Pathomorphology, Medical University of Bialystok, Poland

INTRODUCTION: Crohn's disease and ulcerative colitis a characterized by recurrent remission and flare-ups, and the presence of chronic inflammatory infiltrates even in the absence of clinical manifestations. Simultaneously, changes of the intestinal mucosal barrier are associated with the pathogenesis of inflammatory bowel disease (IBD).

MATERIAL AND METHODS: In the present study we investigate the expression of β-catenin and E-cadherin in normal (n = 6) and inflamed intestine of patients with Crohn's disease (14 patients) and ulcerative colitis (50 patients).

RESULTS: E-cadherin and β-catenin were strongly and evenly expressed by the epithelium in all specimens of intestine studies. Although in some cases of Crohn's disease we observed lower expression of E-cadherin for groups of glands with characteristic morphology. The decrease of E-cadherin expression can be associated with the inflammatory process.

Correspondence:
Katarzyna Guzinska-Ustymowicz MD, PhD
ul. Waszyngtona 13
PL-15-889 Bialystok
Poland
E-mail: kguzinska@poczta.onet.pl
Treatment of distal ulcerative colitis with medication

Stoynov, S., H. Kadian, A. Chavoushian*
Clinic of Gastroenterology, UH "Tzaritza Joanna" Sofia, Bulgaria, *Clinic of Gastroenterology, 5th City Hospital, Sofia, Bulgaria

Treatment has been applied to 97 patients with distal ulcerative colitis (19 - proctitis and 78 - proctosigmoiditis) with mild and moderate activeness of the disease on the background of p.o. therapy with Salofalk® 3 g applied to 27 patients and Salazopyrin 2-4 g - to the other patients. Due to the unsatisfactory response to the p.o. therapy after 45 days the following medications were included in the treatment: Methylprednisolone 40 mg in enemas - 53 patients; Salofalk® enemas 4.0 g/60 ml - 23 patients; Lidocain 800 mg in enemas 3 t.i.d. and Sucralfat 4 g/day.

Among the patients treated with methylprednisolone after 20 days of treatment clinical and endoscopic improvement was reported in 41 patients - 78%. In the group for local treatment with Salofalk® after 28 days clinical and endoscopic improvement was reported in 17 patients - 74%. No adverse effects were reported in the group with Salofalk®, while in the group with methylprednisolone acne was noticed in 2 patients and tenseness/restlessness - in 1 patient.

Conclusion: Local therapy with Salofalk® has good effect, good tolerance, there is practical convenient form for application and there are no adverse effects.
Cathodic stripping voltammetric determination of total selenium content of plasma in inactive Crohn’s disease and ulcerative colitis

Taba Gabriella¹, Blázovics Anna¹, Kovács Ágota², Lado Cristina³, Rapavi Erika¹, Váli László¹, Szentmihályi Klára¹,⁴
¹Semmelweis University, ²Péterfi Sándor Hospital, ³Budapest University of Technology and Economics, ⁴Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary

Selenium depletion may occur in several gastrointestinal diseases when the selenium absorption is impaired. Since the selenium determination in the body fluids is rather complicated because of the very low concentration and the long term chemical methods (atomic absorption spectrometry, etc), the aim of our study was to determine total selenium concentration in plasma of two types of IBD (Crohn’s disease: CD; ulcerative colitis: UC) by a relatively new, sensitive and simple stripping voltammetric technique.

14 Caucasian volunteers, 28 UC and 13 CD patients were investigated. The patients were treated with standard therapy recommended by WHO. Total selenium concentration of plasma was examined in patients with CD and UC. Selenium in plasma was determined with cathodic stripping voltammetry on hanging mercury drop electrode.

The technique applied may be one of the most significant method for selenium determination in body fluids with the lowest detection limit (0.2 ppb), economical running without sample using up.

Significant difference (p < 0.05) was found between the concentration of selenium in plasma of CD (25.32 ± 16.38 ppb) and control group (110.98 ± 44.08 ppb). The selenium content of UC group (106.52 ± 44.34 ppb) showed no difference compared to control patients.

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Long-term efficacy of 6-thioguanine in patients with inflammatory bowel disease

Teml A, Dejaco C, Miehsler W, Harrer M, Vogelsang H, and Reinisch W.
Department of Internal Medicine IV, Division of Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria

Introduction: The aim of the present study was to investigate retrospectively the long-term efficacy of 6-thioguanine (6-TG) in patients with IBD previously intolerant or resistant to azathioprine (AZA) and/or 6-mercaptopurine (6-MP).

Methods: 69 patients with Crohn’s disease (CD; 50/69, 72%), ulcerative colitis (UC; 14/69, 20%) and indeterminate colitis (IC; 5/69, 7%), respectively, were included for induction and maintenance of remission in chronic-active disease. Remission and relapse were defined by the modified Harvey-Bradshaw score in CD, and the clinical activity index in UC and IC, respectively, and the daily steroid requirement. Adverse events (AE) were recorded.

Results: 40/69 patients (58%) reached initial remission after a median time of 114 days (interquartile range [IQR] 54 to 192) and a median 6-TG dose of 40 mg/d (range 10 to 60). Of those, 13 patients (33%) suffered a relapse after a median time of 393 d (IQR 96 to 520). 21/32 of patients (66%) continuing 6-TG treatment are relapse free for a period of 503 d (IQR 128 to 713) at a daily 6-TG dose of 20 mg (range 6 to 60). Therapy was discontinued in 37 patients due to an AE (24/37, 65%), therapy resistance (8/37, 22%), safety concerns (1/37, 3%) and other reasons (4/37, 11%), respectively.

Conclusion: 6-TG is efficient as maintenance and steroid sparing agent in patients with IBD previously intolerant or resistant to AZA/6-MP. A follow-up program for the potential hepatotoxicity of 6-TG is mandatory and has been initiated at our site.
Liver proteosynthetic function and acute phase-proteins in patients with Crohn’s disease during anti-TNF-antibody therapy

L. Turecký, V. Kupcová, Z. Detková, M. Príkazská, E. Uhlíková
Medical School, Comenius University, Bratislava and Institute of Preventive and Clinical Medicine, Bratislava, Slovakia

Crohn’s disease (CD) is a multifactorial disease characterized by chronic intestinal inflammation. In CD, a broad range of cytokine-based therapies are currently being tested, but the anti-TNF approach remains the most effective. The chimeric anti-TNF IgG1 antibody infliximab (Remicade®) has been proven in multiple clinical trials, to be an effective and well tolerated therapy for the management of acute CD.

The aim of our study was to estimate the effect of therapy with anti-TNF on markers of proteosynthetic function of the liver in patients with CD.

Patients and methods: 15 patients with active, refractory CD were studied. Patients were treated with a single infusion of infliximab (5 mg/kg body weight). Blood specimens were collected before and 4 weeks and 4 months after infusion of infliximab. Prealbumin, albumin, transferrin and cholinesterase were estimated in patient’s plasma. The levels of alpha-1-acid glycoprotein were used as marker of inflammatory activity.

Results: Clinical improvement was observed in our patients after infliximab treatment with significant decrease in CDAI from 236 ± 34 before treatment to 132 ± 46 one month after administration of infliximab. There were significant decreased levels of markers of liver liver proteosynthetic function - (patients vs. controls: prealbumin 330 mg/l vs. 222 mg/l, albumin 43 g/l vs. 34.7 g/l, transferrin 3480 mg/l vs. 2480 mg/l, cholinesterase 4500 U/l vs. 3350 U/l). There was significant improvement of plasma levels of these parameters in patients with CD after one month after infusion of infliximab (prealbumin 222 mg/l vs. 280 mg/l, albumin 34.7 g/l vs. 38.5 g/l, transferrin 2480 mg/l vs. 2720 mg/l, cholinesterase 3350 U/l vs. 3730 U/l). The level of alpha-1-acid glycoprotein was decreased after therapy (1130 mg/l vs. 815 mg/l). The increase of liver proteosynthetic function could be the result of decreased inflammatory activity and/or the result of improvement of absorption of amino acids in small intestine. The different answer of both groups of liver proteins is more probably the result of change of interleukine stimulation of hepatocytes during therapy.

Correspondence:

Prof. Ladislav Turecky, MD, PhD
Medical School
Comenius University
Sasinkova 2
SLO-81372 Bratislava
Slovakia
Sonographic prevalence of liver steatosis and biliary tract stones in patients with inflammatory bowel disease

Daniela Tuta, S. Sandulescu, Viorela Enachescu, C. Efrem, Simona Micu
Clinical Hospital of Emergency, Department of Internal Medicine, University of Medicine and Pharmacy of Craiova, Romania

**Background:** Up to 60% of the patients with inflammatory bowel disease have extraintestinal manifestations. Crohn’s disease and ulcerative colitis are associated with pathologic findings in the liver and biliary tract. Ultrasonography (US) represents a noninvasive means to study hepatobiliary abnormalities.

**Aim:** This study evaluated the prevalence of US hepatobiliary changes and their relationship to clinical variables in the patients with inflammatory bowel disease

**Patients and methods:** 18 patients with Crohn's disease (average age = 38, 12 women and 6 men), 24 patients with ulcerative colitis (average age = 40, 14 women and 10 men) were studied using US. There were recorded the size of the liver, its echogenicity (graded as mild-to-moderate or severe indicating a corresponding degree of hepatic steatosis), the focal lesions of the liver and gallbladder and biliary tract abnormalities.

**Results:** Hepatobiliary abnormalities were found using US in 10 patients with Crohn's disease and 12 patients with ulcerative colitis. Liver enlargement and mild-to-moderate to severe liver steatosis were found in 6 and 8 patients with Crohn's disease and in 6 and 10 patients with ulcerative colitis, respectively, but two patient with Crohn's disease and four patients with ulcerative colitis suffered from metabolic disorders or obesity. Gallstones were found in 4 patients with Crohn's disease and 6 patients with ulcerative colitis.

**Conclusion:** The prevalence of liver enlargement, liver steatosis and gallstones stones was higher among patients with inflammatory bowel disease.

**Correspondence:**

Daniela Tuta
Dacia Street, bl. N1, ap. 15
RO-200066 Craiova
Departement of Dolj
Romania
Telephone: +0040745825578
E-mail: tdl17tdl@yahoo.com
The efficiency of novel biological therapies in severe inflammatory Crohn's disease: Our experience

R. Vadan, L. Gheorghe, M. Badea, C. Angelescu, S. Constantinescu, I. Calin, T. Marinescu, C. Gheorghe, M. Diculescu
Gastroenterology and Hepatology Center, Fundeni Clinical Institute, Bucharest, Romania

Background and aim: The therapy of moderate/severe Crohn's disease (CD) has recently been the subject of significant advances with the introduction in practice of biological therapies. The first agent with proven efficiency was Infliximab, an anti TNF alfa chimeric antibody. Our aim was to evaluate the response and the tolerance to Infliximab in CD patients with severe disease.

Methods: We evaluated all the patients admitted in our unit with severe inflammatory CD, which did not respond to intravenous corticotherapy and received Infliximab (5 mg/kg, once) to achieve remission. The following data were recorded: age (current and at diagnosis), sex, smoking habits, duration of the disease, the presence of extraintestinal manifestations, previous treatment. CDAI was calculated at inclusion and after 2 weeks, a reduction with > 70 points defined the partial response and a value < 150 the complete response.

Results: 26 patients were included: 14 males, 12 females, mean age 36.8 ± 2.12 years. From these, 76.92% responded to the treatment, 57.69% with complete response and 19.23% with partial response, while 23.08% did not respond. The age, sex, duration of the disease and the other parameters evaluated did not differ between responders and non-responders. The infusion reactions were recorded in 19.22% (five cases), 15.38% were mild-fever, myalgias, headache, nausea and 3.84% (one patient) severe-sepsis.

Conclusion: Infliximab is a highly effective and safe therapy for the induction of remission in patients with severe CD that do not respond to other therapies. The availability of biological therapies opens a new era in the therapeutic approach of CD.
Overexpression of indoleamine 2,3-dioxygenase in human inflammatory bowel disease

Anna Maria Wolf, Dominik Wolf, Holger Rumpold, Alexander R. Moschen, Arthur Kaser, Peter Obrist, Dietmar Fuchs, Gerald Brandacher, Christiane Winkler, Karel Geboes, Paul Rutgeerts and Herbert Tilg
Division of Gastroenterology and Hepatology, Division of Hematology and Oncology, Institute of Pathology, Institute for Medical Chemistry and Biochemistry, Department of General and Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria
Ludwig Boltzmann Institute of AIDS-Research, Innsbruck, Austria
Department of Pathology, Department of Gastroenterology, University of Leuven, Belgium

T-cells are causally involved in the pathogenesis of inflammatory bowel disease (IBD). The tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) regulates T-cell proliferation and survival. We show in this report that IDO mRNA is markedly induced in lesional colonic biopsies of IBD patients. IDO is primarily expressed in CD123+ mononuclear cells infiltrating the submucosal areas of the inflamed lesions. In Crohn's disease (CD), IDO is also strongly expressed in perifollicular regions of lymphoid follicles. Upregulation of IDO is of functional significance, as we detected an increase of kynurenine and of the kynurenine/tryptophan ratio in supernatants from colonic explant cultures (CECs) of CD patients. Immunohistochemistry of colonic biopsies taken from CD patients prior and after treatment with the TNF-blocking antibody Infliximab revealed reduced IDO expression in patients with good clinical response to Infliximab. In summary, high local expression of IDO may represent an anti-inflammatory mechanism tempting to counterbalance the tissue-damaging effects of activated T-cells infiltrating the colonic mucosa in IBD.
The influence of partial gastrectomy on the ultrastructural changes in the liver - An experimental animal model

Department of General, Gastroenterological and Endocrinological Surgery, *Department of Clinical Pathomorphology of the Karol Marcinkowski University School of Medical Sciences Poznan, Poland

The aim of our studies was to evaluate the ultrastructural changes in the liver in rats after partial gastrectomy. The studied group consisted of 19 Wistar strain rats. In 15 animals partial gastrectomy was done in Billroth I (n = 5), Billroth II (n = 5) or Y-Roux (n = 5) mode. The control group underwent laparotomy, only. In 4, 8 and 12 months after gastrectomy animals were anestesied with ether and then the liver biopsies were taken during laparotomy. All tissue material was fixed and then prepared in a routinous way for histological and electron microscopy studies.

During histological examinations no abnormalities were found. On the ultrastructural level (in tissue samples taken from animals, no more then 4 months after gastrectomy we found mitochondrial degeneration, vacuolization of cytoplasm, abnormalities in the nuclei and increased activity of B-K cells. The focal necrosis of the hepatocytes was also observed. In tissue samples from animals after 4 to 12 months after primary operation we found increasing number and size of lipid vacuoles in the cytoplasm of hepatocytes The number of Ito cells increased, also. There was no differences between animals operated in Billroth I, Billroth II or Y-Roux mode.

The gastrectomy caused only minimal ultrastructural changes in the liver tissue within the period of observation. Those changes describe above may result from numerous metabolic derangements found after gastrectomy.
Chinese Beiqishen tea influences the redox-homeostasis of liver and gut

Anna Blázovics¹, Andrea Lugasi², Éva Stefanovits-Bányai³, É. Héthelyi³, Erika Rapavi¹, Andrea Balázs¹, Klára Szentmihályi⁴

Semmelweis University¹, National Institute of Food Hygiene & Nutrition², Corvinus University³, Hungarian Academy of Sciences⁴, Budapest, Hungary

Pro-inflammatory cytokines, apoptosis signalling and redox-response transcription factors are depended on free radicals and trace elements. Prostaglandins have pro- and anti-inflammatory effects on the immune system. Teas are important flavonoid sources therefore Chinese Beiqishen tea was studied in in vitro and in vivo redox test systems. Proton-donating ability, reducing power, and scavenger capacity were identified in liver and gut of healthy and hyperlipidemic Wistar rats. Metal element content of the liver tissue was measured as well. Serum parameters were also determined.

On the basis of our examinations, it may be established that the predominant compound for refreshing effect of Beiqishen tea is caffeine. The enormous polyphenol and tannin contents are unfavorable as well as polluting toxic metal elements accumulated in the liver and eliminated mainly with the bile. High concentrations of active antioxidant components and toxic elements of tea extract influenced the redox-homeostasis, increased the oxidative stress and peroxidation processes. Caffeine and toxic metal elements, especially As, Cr, Ni and Fe have opposite effects to those of phenolic (quercetin, kaempherol) antioxidants.

Conclusions: Our results draw attention to the fact that the consumption of different herb extracts without efficient quality and/or content can cause serious damages to health.

This study was supported by ETT-002/2003 and NKFP-1/016 grants.
Hepatic steatosis in chronic virus C infection

Dr. Gabriel Coltan**, Dr. F.A. Caruntu**, Dr. Cristina Marin**, Dr. Angela Manolache*
**National Infectious Diseases Institute "Prof. Dr. Matei Bals", Bucharest, Romania
*Military Hospital Craiova, Infectious Diseases Department, Romania

**Background:** The prevalence of steatosis is 30-70% in patients chronically infected with HCV, which is higher than that observed in other forms of chronic liver disease. It is incriminated the causative role of HCV in the pathogenesis of steatosis and a permissive role of steatosis in HCV persistence.

**Patients and methods:** We have studied the histological examination of the liver biopsy, in 217 patients with antiviral therapy hospitalized during 2003-2004 period, in our clinic. The steatosis degree was settled on METAVIR score, as well as based on the histological description (aspect, distribution) of the liver fragment. We have put together histological examination data, clinical examination, laboratory and imagistic investigations, for highlighting associated liver diseases.

**Results:** Among 217 patients of the actual study, 59 of them presented steatosis at liver biopsy. Steatosis was minimal in 50 patients and moderate/severe in 9 patients.

**Conclusions, discussions:**
1. Hepatic steatosis is frequent in Romania in C chronic hepatitis, despite the present C genotype: 1b.
2. Incidence is less important compared with international studies.
3. Men are more likely to develop steatosis compared to women, ratio 2:1 (39 M, 20 F).
4. One third of patients with hepatic steatosis had associated other diseases (mainly obesity) that could influence or trigger steatosis.
Non-alcoholic fatty liver disease: Clinical and histological aspects

Damian Dana, Berecz Andrea, Fetica Iulia, Grigorescu Mircea, Serban Alexandru
3th Medical Clinic, Cluj Napoca, Romania

Non-alcoholic fatty liver disease is a hepatic disorder of growing importance in the Western countries and is associated with the increasing prevalence of the metabolic syndrome. The present study evaluates the clinical and histological features of biopsy-proven non-alcoholic fatty liver disease and investigates the predictors of severe histological disease in a group of Eastern European patients.

Methods: Clinical data from 33 patients, with a histological diagnosis of NAFL, admitted in our department between 2002 and 2004 were reviewed retrospectively, after exclusion of other causes of liver disease. We analyzed the demographical, clinical, laboratory and histological parameters of these patients.

Results: From the 33 patients 24 (73%) were male and 9 (27%) were female. The median age was 42 ± 8 years. 64% had elevated BMI (27% were obese and 36% were overweight). 85% had dyslipidemia, 48% had both cholesterol and triglyceride levels elevated, 33% had diabetes mellitus. The median ALT level was 90 ± 49 U/l. Most of the patients had steatohepatitis (the elevated ALT level being the main indication of liver biopsy), 62% had severe steatosis, 29% had significant fibrosis.

Conclusion: The prevalence of NAFL was higher in male patients. Most patients with NAFL had features of metabolic syndrome. We found no correlation between biochemical markers and histological severity of NAFL. Diabetes mellitus (especially with insulin need) was the most important predictor of severe histological disease.
The xenobiotic transporters Mrp2 (Abcc2) and Bcrp (Abcg2): Basal expression in intestine and liver, regulatory events and their mechanisms in obstructive cholestasis are gender-specific

Christoph G. Dietrich, Carsten Gartung, Siegfried Matern, Andreas Geier
Dept. of Gastroenterology and Hepatology, Med. Clinic III, Aachen University, Germany

Introduction: Toxic effects of xenobiotics have been shown to be gender-dependent (Ghandi, Ann Rev Pharm Tox 2004). We analyzed differences in the basal expression and regulation of the ABC-transporters Mrp2 and Bcrp between male and female rats during obstructive cholestasis.

Methods: After one week of bile-duct ligation in rats of both gender we obtained small intestine and liver from all animals. Sham-operated rats served as controls. Western and Northern blots and EMSA were used to determine protein mass, mRNA concentrations and binding of gene transactivators.

Results: Basal protein expression of Mrp2 was not gender dependent in liver or intestine. Female hepatic Bcrp expression was only 68 (4) %, however intestinal Bcrp expression amounted to 116 (6) % of male rats. Intestinal Bcrp downregulation was significantly more pronounced in female rats (40 [13] % vs. 69 [11] % of controls). In contrast, downregulation of both intestinal and hepatic Mrp2 was significantly more sustained in male rats than in female rats (intestine 17 [4] % vs. 70 [15] % of controls, liver 15 [6] % vs. 45 [7] % of controls). Northern blots revealed transcriptional mechanisms for regulation. EMSA with rat Mrp2 gene regulatory elements identified RXRα:RARα as the responsible transactivator for gender-specific downregulation of Mrp2.

Conclusions: Differences in protein expression in intestine and liver between genders comprise basal expression (Bcrp) and regulatory events (Mrp2), the latter mediated by RXRα:RARα. Our results confirm widespread gender-dependent mechanisms in transporter gene regulation which may influence bioavailability and metabolism of xenobiotics in a gender-specific fashion.
Aspects of lipoproteins metabolism associated with non-viral/non-alcoholic liver steatosis (NV/NALS)

Mihaela Dinca, Viorela Enachescu
University of Medicine and Pharmacy Craiova, Romania

Introduction: NV/NALS is mainly associated with obesity and diabetes mellitus, both affections being presently accepted as cardiovascular risks.

Aim: To evaluate lipoproteins metabolism at patients diagnosed with NV/NALS.

Material and method: The investigated group included 116 subjects (52 women, 64 men), aged between 30 and 60, having associated hepatomegaly, moderate aminotransferases increase and gamma-glutamyltransferases, excluding the viral etiology, immune or alcoholic, remaining only the NV/NALS presumption. Also were determined the Body Mass Index, Waist Hip Ratio, total cholesterol (T-Ch), its fractions (HDL-Ch, LDL-Ch, VLDL-Ch) and plasmatic triglycerides, using for interpretation the FRIEDERICKSON classification.

Results: The presence or the absence of abdominal obesity (AO) divided the subjects into two sets: Set A, 84 subjects (40 women, 44 men) with AO and Set B, 32 subjects (12 women, 20 men) without AO. Set A subjects presented the following lipid profiles: Women - normal, 10%; IIa-type, 10%; IIb-type, 15%; IV- type, 45% out of which 55% associated hypo-HDL-Ch; isolated hypo-HDL-Ch, 20%; Men - normal, 9%; IIa-type, 13.6%; IIb-type, 20.4%; IV-type, 41% out of which 69% associated hypo-HDL-Ch; isolated hypo-HDL-Ch, 16%. Set B subjects presented the following lipid profiles: Women - normal, 25%; IIa-type, 33.4%; IIb-type, 25%; IV-type, 16.6%; Men - normal, 25%; IIa-type, 35%; IIb- type, 20%; IV-type, 20%.

Conclusions: NV/NALS was associated with AO at 72.4% subjects (76.9% women, 68.7% men). The lipid profile, associated with NV/NALS, is of atherogenic type, predominant with VLDL-Ch, frequently associating hypo-HDL-Ch. NV/NALS may be considered a clinical marker of cardiovascular risk for patients with AO.

Correspondence:

Mihaela Dinca, MD, PhD
RO-1100 Craiova
Calea Bucuresti, no. 37, bl. 21E, ap. 14
Romania
Phone: +40 251 415030
Fax: +40 251 415030
E-mail: adrsv@topedge.ro
Hepatic endothelial CCL25 mediates the recruitment of CCR9⁺ gut homing lymphocytes to the liver in primary sclerosing cholangitis

Bertus Eksteen, Allister J. Grant, Alice Miles, Stuart M Curbishley*, Patricia F Lalor, Stefan G Hübscher, Michael Briskin‡, Mike Salmon* and David H. Adams*

*Liver Research Laboratories and *Rheumatology, Institute for Biomedical Research, University of Birmingham, Birmingham, B15 2TT, UK
‡Millennium Pharmaceuticals Inc, 75 Sidney St, Cambridge, MA 02139, USA

Primary sclerosing cholangitis, a chronic inflammatory liver disease characterized by progressive bile duct destruction, develops as an extra-intestinal complication of inflammatory bowel disease. However, the liver and bowel inflammation are rarely concomitant and PSC can develop in patients whose colons have been previously removed. We hypothesized that PSC is mediated by long-lived memory T-cells originally activated in the gut but able to mediate extra-intestinal inflammation in the absence of active IBD. In support of this we show that liver-infiltrating lymphocytes in PSC include mucosal T-cells recruited to the liver by aberrant expression of the gut-specific chemokine CCL25 which activates α₄β₇ binding to MAdCAM-1 on hepatic endothelium. This is the first demonstration in humans that T cells activated in the gut can be recruited to an extra-intestinal site of disease and provides a paradigm to explain the pathogenesis of extra-intestinal complications of IBD.
Biliary epithelial injury is associated with CCL28 production and the recruitment of CCR10 effector lymphocytes in human chronic hepatitis

*Liver Research Laboratories, Institute for Biomedical Research, University of Birmingham, Birmingham, B15 2TT, UK
*Millennium Pharmaceuticals Inc, 75 Sidney St, Cambridge, MA 02139, USA

CCL28 is constitutively expressed by epithelial cells at several mucosal sites, including the colon, where it is believed to play an important role in immune homeostasis by recruiting mucosal lymphocytes and IgA producing plasmablasts. Increased expression of CCL28 by pro-inflammatory cytokines and bacterial products would support a wider role for CCL28 in recruiting inflammatory cells to areas of epithelial injury. Chronic hepatitis is characterised by a heavy lymphocytic infiltrate in the portal tracts which in biliary diseases is associated with lymphocytic infiltration and destruction of biliary epithelium. We report that CCL28 is expressed by biliary epithelium in cholestatic liver diseases during biliary injury. Stimulation of cultured primary human biliary epithelial cells with IL-1β, LPS and bile acids increased production of CCL28 significantly. The receptor for CCL28, CCR10 was detected on liver-infiltrating lymphocytes with the highest levels detected in the biliary diseases primary sclerosing cholangitis and primary biliary cirrhosis where bile duct inflammation predominates. CCR10 expression was confined to a population of CD11a^high/^CD45RO+ effector lymphocytes. Exposure of CCR10 liver-infiltrating lymphocytes to CCL28 induced adhesion to either VCAM-1 or MAdCAM-1. Our findings suggest that CCL28 expression by biliary epithelial cells may be important in recruiting effector cells to bile ducts and thus promoting bile duct injury.
Overlap syndrome and atypical forms of autoimmune hepatitis: Our experience

V. Enachescu, M.E. Dinca
III and II Medical Clinic, Clinical Hospital no. 2, University of Medicine and Pharmacy of Craiova, Romania

Background: Studies in the literature have remarked the different response to treatment and evolution of different types of autoimmune hepatitis.

Aim: The comparative analysis of the types of autoimmune hepatitis in order to individualize treatment and to establish prognosis.

Material and methods: A retrospective study performed on 14 cases (10 females, 2 males). Diagnosis was established using the 1999 score of the International Study Group.

Results: Distribution of cases: type I autoimmune hepatitis (AIH) 6 cases, type II AIH 1, AIH/primary biliary cirrhosis (PBC) 2, AIH/HCV 3, AIH/rheumatoid poliarthritis 1, AIH/systemic erythematous lupus 1 case. One case presented acute severe debut. Associated conditions: autoimmune hemolytic anemia 1 case, thyroiditis 2, kidney affection 3, diabetes mellitus 2 cases. We remarked positive HCV-RNA with anti-LKM1 antibodies in 3 cases. Immunophenotypation showed Th/Ts = 4.8 in type I AIH and Th/Ts = 4.1 in type II AIH. The therapeutic management was different: type I AIH: azathioprine + budesonide 2 cases, metyldprednisolone 1 case, prednisone 3 cases, with favorable response in 50%. Type II AIH: azathioprine + prednisone, with favorable response; AIH/HCV: interferon followed by CellCept; AIH/PBC: prednisone + Ursofalk®, with favorable response in all cases.

Conclusions: 1. Type I AIH and AIH/HCV prevail. 2. Overlap syndromes respond favorably to immunosuppressive therapy. 3. Extrahepatic manifestations are more important and more severe in atypical cases, with renal affection that may even lead to chronic renal failure.

Correspondence:
Viorela Enachescu
Stirbei Voda street, nr. 16
RO-200374, Craiova
Departament of Dolj
Romania
Phone: + 0040251533516
E-mail: vemd1@yahoo.com and vemd1@umfcv.ro
Cardiac abnormalities in patients with cirrhosis

Carmen Fierbinteau-Braticevici, Andreea Bengus, Maria Udeanu, D. Andronescu
Medical Clinic II, Department of Gastroenterology, University Hospital Bucharest, Romania

Cirrhotic patients have asymptomatic cardiac abnormalities that may predict sudden death.

AIM: To assess the cardiovascular risk of cirrhotic patients and the possible pathogenetic mechanism.

METHODS: Eighty cirrhotic patients without overt heart disease were studied. They underwent ECG (Q-T interval), echocardiography (left ventricular function) and neurohumoral activity (plasma renin activity, serum aldosterone, and urinary methanephrines). Serum creatine kinase MB, myoglobin and cardiac troponin concentrations were measured.

RESULTS: Q-T interval was prolonged (> 430 ms) in 48 patients (60%). Q-T length was correlated with the etiology and the severity of cirrhosis and with biological and echographical parameters. Multivariate analysis showed that high Child- Pugh score (p < 0.05), elevated urinary methanephrines (p < 0.001), elevated serum cardiac troponin (p < 0.002) were correlated with Q-T duration and with subclinical left ventricular myocardial damage.

CONCLUSIONS: QT interval is frequently prolonged in cirrhotic patients regardless the etiology of the disease. It is associated with the severity of the disease and with sympathoadrenergic hyperactivity that induced asymptomatic myocardial damage. Beta-blocker treatment may protect the cirrhotic patients against the high cardiovascular risk.
Role of ursodeoxycholic acid against oxidative stress in primary biliary cirrhosis

Carmen Fierbinteănului-Braticevici, P. Dragomir, D. Andronescu
Medical Clinic II, Department of Gastroenterology University Hospital Bucharest, Romania

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that may lead to liver failure. Ursodeoxycholic acid (UDCA) improves clinical and biochemical indices and delays disease progression.

Aim: To evaluate the role of UDCA against oxidative stress and to assess the possible role of oxidative stress in the pathogenesis of PBC.

Methods: Forty patients with proven PBC were clinical, biological and histological evaluated at the beginning of the study and 3 months after UDCA treatment. We associated histological necroinflammatory activity with the oxidative stress. The oxidative stress was evaluated by the levels of serum malondialdehyde (MDA) and serum total glutathione (GSH).

Results: The severe necroinflammatory activity was strongly correlated with the increase of lipid peroxidation (MDA > 250 nanomol/dl) and a decrease of GSH (< 40 μmol/dl). Compared with pretreatment values, serum MDA decreased and GSH increased significantly (p < 0.001) along with clinical, histological and biochemical improvement observed after UDCA treatment.

Conclusions: The degree of hepatic inflammation in PBC generated proportionally free radicals which induce oxidative stress. The cytoprotective mechanism of UDCA may be mediated by protection against oxidative stress. The association of UDCA with new antioxidant drugs seems beneficial in PBC treatment.
Cytokine-independent repression of rodent Ntcp in obstructive cholestasis

Andreas Geier¹, Gernot Zollner², Christoph G. Dietrich³, Martin Wagner², Peter Fickert², Helmut Denk³, Nico van Rooijen⁴, Siegfried Matern¹, Carsten Gartung¹, and Michael Trauner²

¹Department of Internal Medicine III, Aachen University (RWTH), Aachen, Germany, ²Division of Gastroenterology and Hepatology, Department of Internal Medicine, ³Department of Pathology, Medical University Graz, Graz, Austria, and ⁴Department of Cell Biology, Vrije University, Amsterdam, The Netherlands

Background: Cholestatic liver injury is a complex pathophysiologic situation not only associated with accumulation of bile acids but also with activation of proinflammatory cytokines. Common bile duct ligation (CBDL) induces sustained downregulation of the Na⁺/taurocholate cotransporter (Ntcp) in rat and mouse liver. While repression of Ntcp during endotoxemia is cytokine-mediated (Geier et al. Hepatology 2003), it is unclear whether inflammatory cytokines contribute to this down-regulation in obstructive cholestasis.

Methods: Cytokine inactivation in CBDL rats and mice was either performed directly with TNFα- (etanercept 8 mg/kg i.p.) or IL-1β-inactivation (anakinra/AMG 719 100 mg/kg i.p.) or indirectly via Kupffer cell depletion by i.p.-administration of 4 ml/kg liposome-encapsulated dichloromethylene-bisphosphonate. Protein and mRNA expression of Ntcp and the orphan nuclear receptor SHP were analyzed by Western and Northern blotting. Key regulatory transcription factors for Ntcp (HNF1α, HNF4α, RXRα:RARα) were studied by EMSA and nuclear Western analysis.

Results: Both methods of cytokine inactivation failed to maintain Ntcp protein or mRNA expression within 3 days after CBDL in either rats or mice (20-40% of sham-controls) while SHP mRNA expression remained increased 3- to 5-fold. Decreased nuclear HNF1α and HNF4α (45% and 60% of sham-controls, respectively) as well as HNF1α binding activity (32% of sham-controls) were not restored during cytokine inactivation after CBDL, indicating cytokine-independent mechanisms of Ntcp regulation. RXRα:RARα binding remained unchanged in all experimental conditions.

Conclusion: Our findings support a new model of distinct transcriptional pathways involved in the regulation of Ntcp expression during cholestasis depending on the type of cholestatic liver injury. During obstructive cholestasis accumulating bile acids per se without major contribution of cytokines lead to downregulation of Ntcp via repression of HNF1α and HNF4α.
Liver steatosis and inflammatory bowel disease

Amelia Genunche-Dumitrescu, Simona Popa, P. Ciurea, P. Mitrut, Daniela Badea
University of Medicine and Pharmacy, Craiova, Romania

Hepatobiliary lesions are frequently associated with inflammatory bowel disease. The most commonly reported syndroms are chronic hepatitis, steatosis, cirrhosis and primary sclerosing cholangitis.

Liver steatosis has various etiologies: obesity, alcohol, diabetes mellitus, metabolic disorders, drugs.

**Aim:** To evaluate the prevalence of liver steatosis among inflammatory bowel disease patients.

**Material and methods:** We included in this study 88 patients (56 females and 32 males) with inflammatory bowel disease. According to the clinical picture, laboratory examinations, colonoscopy with histopathological examinations, 54 patients had ulcerative colitis and 34 patients had Crohn's disease. Routine ultrasound was performed using a 3.5 MHz transducer. At ultrasound, liver size, echogenity, focal lesions of the liver and biliary tract abnormalities were recorded. Statistical analysis was performed using Wilcoxon and Kruskal-Wallis Tests with p-values < 0.05 considered statistically significant.

**Results:** Mean age of both groups was similiary: 42.12 years for patients with ulcerative colitis and 38.98 years for patients with Crohn's disease (p = 0.039). Liver steatosis was present in 43 patients, but 12 patients (13.64 % of the group) had other steatosis etiologies: diabetes mellitus type II (4 patients), alcoholism (2 patients), obesity (3 patients), dyslipidemia and other metabolic disease (3 patients). Liver steatosis was present in 21 patients of ulcerative colitis and 10 patients with Crohn's disease.

**Conclusion:** The prevalence of liver steatosis was increased in ulcerative colitis patients.
Comparative study regarding the risk factors of NASH and NAFLD in high-risk population

A. Goldis, D. Lazar, C. Vernic, R. Goldis, V. Lungu, V. Serban*
Gastroenterology Department, *Metabolic Diseases Department, County Hospital Timisoara, Romania

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are often underevaluated as evolutive severity and prevalence, even if data from the literature suggest a 10% of evolution to cirrhosis. The aim of the study was to investigate in high-risk population the prevalence of NASH and NAFLD and the influence of some possible risk factors.

Material and method: We prospectively investigated a batch of 1023 patients considered with high-risk (diabetes, obesity or hyperlipemia) hospitalized in the department of metabolic diseases. All the cases were investigated by ultrasononography for steatosis and laboratory tests. We excluded the cases with viral or alcoholic etiology. Only cases with NAFL and NASH were studied.

Results: The final batch included 522 (51%) patients with NAFLD: 444 (43%) NAFL and 78 (7.6%) NASH:

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<th>NAFL</th>
<th>NASH</th>
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<tr>
<td>BMI</td>
<td>30.75 ± 5.52</td>
<td>31.22 ± 6.65</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>247.96 ± 141.70</td>
<td>300.4 ± 171.3</td>
<td>0.004 (ES)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>224.00 ± 69.91</td>
<td>233.75 ± 59.44</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin dose</td>
<td>41.73 ± 26.47</td>
<td>48.50 ± 34.20</td>
<td>0.0476 (S)</td>
</tr>
<tr>
<td>Insulin dose/kg</td>
<td>0.9 ± 0.89</td>
<td>0.99 ± 0.92</td>
<td>NS</td>
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<tr>
<td>Steatosis (degree)</td>
<td>1.37 ± 0.74</td>
<td>1.43 ± 0.69</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>57.18 ± 0.99</td>
<td>55.61 ± 11.9</td>
<td>NS</td>
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Conclusions: In our study, subjects with NASH had a significant increase of triglyceridemia and insulin dose compared with the subjects with NAFL. The prevalence of NASH and NAFLD in patients with high-risk was very high (7.6% and 51%).
Non-alcoholic steatohepatitis (NASH) in the general population of Western Romania

Ramona Goldis, Daniela Lazar, C. Tudor, V. Lungu, A. Goldis
Gastroenterology Department, County Hospital Timisoara, Romania

Nonalcoholic fatty liver disease (NAFLD) and NASH are two entities with increasing prevalence worldwide. The aim of our prospective study was to detect their prevalence in our region and the association with some possible predisposing factors.

**Material and method:** Our study included a group of 516 persons (253 M, 263 F) from the general population with mean age 50.57 years, in which we investigated the presence of NAFLD and NASH. The diagnosis was made on the ultrasonographic presence of steatosis, in the absence of alcohol consumption or other causes of increased aminotransferases. Patients with NAFLD/NASH were evaluated regarding age, sex and the presence of major risk factors, such as type 2 diabetes, obesity and hyperlipidemia.

**Results:** 123/516 (23.83%) persons presented steatosis at ultrasonography. From these, 24/123 were considered as alcoholic steatohepatitis (ASH) and 99/123 (80.48%) NAFLD – 48 (48.48%) men and 51 (51.52%) women. 21/99 (21.21%) patients presented NASH - 12 (57.14%) men and 9 (42.86%) women.

Prevalence in general population and association with predisposing condition:

<table>
<thead>
<tr>
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<th>NAFLD (n = 99)</th>
<th>NASH (n = 21)</th>
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<tr>
<td>Prevalence</td>
<td>99/516 (19.18%)</td>
<td>21/516 (4.06%)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>23/99 (23.23%)</td>
<td>6/21 (28.57%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>8/99 (8.08%)</td>
<td>2/21 (9.52%)</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>23/99 (23.23%)</td>
<td>5/21 (23.8%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>36/99 (36.36%)</td>
<td>9/21 (42.85%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>26/99 (26.26%)</td>
<td>4/21 (14.04%)</td>
</tr>
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</table>

**Conclusions:** Our region encounters an increased rate of NAFLD and NASH, probably due to the presence of a large number of persons with obesity and hiperlipidemia. The disorder occurs with an approximately equal frequency in men and women, most often in middle-aged persons.
Cholestasis markers before and after cholecystectomy

Gotia Smaranda Rodica¹, Enache Ofelia², Szucsik I. Adalbert²
¹Department of Physiology, and ²Third Surgery Department, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

The clinical trials were carried out in 978 patients with biliary tract disease from Third Surgery Clinic, Timisoara, over a period of four years. 912 patients with acute and chronic cholecistitis presented an infection with local inflammatory response. In 66 patients with complicated cholecistitis (icterus, gangrene) presented severe infections, with systemic inflammatory response.

All patients were investigated for cholestasis markers (serum bilirubin, alkaline phosphatase, cholesterol) before and after cholecystectomy. Serum bilirubin was increased in 279 patients, Alkaline phosphatase was raised in 503 patients (51.43%) with biliary tract lithiasis and reached the normal level after 1-7 days, after surgery.

Cholesterol was raised in all patients with mechanic icterus (74 patients), in 112 patients with acute cholecystitis and in 210 patients with chronic cholecystitis. Cholesterol value lowered in all patients undergoing surgery for mechanic icterus, and in 98 from 322 patients undergoing surgery for cholelithiasis.

Daily rate and the period need to reach the normal level for cholestasis markers depended of the pretreatment serum levels. Desobstructions of the main biliary tract, removing of the mechanical obstacle and of the inflammatory process lead to normal cholestasis markers values. In these patients cholecystectomy was the main therapeutic alternative, preventing the liver further destructions and improving life quality.

Correspondence:

Prof. Dr. Gotia Smaranda Rodica
Department of Physiology
University of Medicine
and Pharmacy "V. Babes" Timisoara
2 Eftimie Murgu street, 300041
RO-1900 Timisoara
Romania
E-mail: smarandagotia@yahoo.com
Hypertransaminasemia in clinical picture of the celiac disease in children

Urszula Grzybowska-Chlebowczyk¹, Halina Wos¹, Sabina Wiecek¹, Maciej Kajor², Maria Szymanska¹

¹Department of Pediatric Silesian Medical University, Katowice, Poland
²Department of Pathology, Silesian Medical University, Katowice, Poland

Introduction: During the last few years we have been observing the decreasing incidence of the symptomatic celiac disease and increasing incidence of mono- and asymptomatic disease. Various atypical symptoms and extraintestinal manifestations were observed mainly in older children but in younger too. The most common is chronic hepatitis and hypertransaminasemia (HT). The aim of the study was to evaluate the prevalence of hypertransaminasemia and the frequency with which HT was the only manifestation of CD.

Materials and Methods: We have evaluated the clinical course of celiac disease in 17 children (14 girls and 3 boys), aged between 12 months to 17 years (mean - 8.2 years). The celiac disease was diagnosed on the grounds of clinical symptoms, histopathological examination of the small intestinal endoscopic biopsy and immunological examinations: serum anti-tranglutaminase antibodies and anti-endomysium antibodies.

Results: Hypertransaminasemia or hepatitis were diagnosed in 8 children (47%), in 4 children aged 12-30 months and in 4 older children. In 4 of them full symptomatic hepatitis (hypoalbuminaemia, dysfunction of the prothrombin system) was the main manifestation of the disease.

Conclusions: Because CD is highly prevalent in the general population, screening for CD should be introduced in the diagnostic procedure in chronic hypertransaminazemia in children.
Successful treatment of Wolman Disease by unrelated umbilical cord blood transplantation

Akiva Hirsch, MD, Jerry Stein, MD, Yael Dror, MA, Eyal Fenig, MD, Ben-Zion Garty, MD, Dimitri Lumelski, MD, Raanan Shamir, MD, Marsha Zeigler, PhD, Batya Stark, Isaac Yaniv MD

BMT Unit, Departments of Pediatric Hematology-Oncology, Clinical Nutrition, Pediatrics, Radiology, and Gastroenterology, Schneider Children's Medical Center of Israel, Department of Oncology, Rabin Medical Center, Department of Pediatric Gastroenterology, Rambam Medical Center, Department of Human Genetics, Hadassah University Hospital, Sackler Faculty of Medicine, Tel Aviv University, Israel

Wolman Disease (WD) is a rapidly fatal lysosomal storage disease caused by the complete absence of acid lipase activity. We report the cure of an infant with WD following transplantation of unrelated HLA-mismatched umbilical cord blood-derived (UCB) stem cells. UCB was chosen as a stem cell source due its immediate availability, its low risk of infection transmission, and its reduced tendency to cause graft vs. host disease. Early transplantation resulted in rapid restoration of normal AL levels before the onset of Wolman Disease-induced, permanent end-organ damage. More than one year after transplant, the patient is thriving and has normal levels of acid lipase in peripheral blood cells. To our knowledge, this is the first report of a successful unrelated cord blood transplant in Wolman Disease.

Key words: in-born error of metabolism, stem cell transplant
Prevalence and clinical significance of endomysial autoantibodies in patients with autoimmune liver disease

Hofer H., Pirker A., Vogelsang H., Gangl A., Penner E., Novacek G.
Department of Internal Medicine IV, Gastroenterology and Hepatology, Medical University of Vienna, Vienna, Austria

Background and Aim: An association between celiac disease (CD) and autoimmune liver diseases such as primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) has been reported. Presence of CD in these patients may further deteriorate clinical status and liver function and predispose to intestinal lymphoma and osteoporosis. Therefore, screening for CD with optional introduction of a gluten-free diet may be of substantial benefit. The aim of the study was to investigate prevalence and clinical significance of endomysial autoantibodies in patients with autoimmune liver disease.

Patients and Methods: So far, 85 consecutive patients have been evaluated. Forty-three patients with PBC (40 women, age: 54.5 ± 11.2, years, mean ± SD) and 42 patients with AIH (35 women, age: 47.0 ± 17.1). Antibodies against endomysium (IgA-EMA, Celikey, Pharmacia) were determined and in EMA-positive patients small bowel biopsies were performed.

Results: EMA were found in two patients with PBC (4.65%, 95% CI: 0-10.9%) without a known history of CD and in one patient with AIH (2.38%, 95% CI: 0-6.9%) with a pre-existing diagnosis of CD. Dietary non-compliance was accompanied by a flare of hepatitis in the AIH patient. Small bowel biopsy revealed normal histology in one PBC patient and was not yet performed in the other.

Discussion: Our preliminary results confirm an increased prevalence of EMAs in PBC and AIH patients. CD might present subclinically and introduction of a gluten-free diet may help to prevent osteoporosis and intestinal lymphoma. In addition, it might improve liver function in AIH patients.
Inborn errors of metabolism presenting as a liver failure

Ewa Jamroz*, Justyna Paprocka*, Jerzy Pietruszewski*, Maciej Adamowicz**, Elzbieta Marszał*
Department of Child Neurology Silesian Medical University, Katowice, Poland
The Children's Memorial Health Institute, Warsaw, Poland

Inborn errors of metabolism may have different effects on the liver depending on the age of the child. Congenital liver failure are typical for GM1 gangliosidosis, Niemann-Pick type A and C, galactosialidosis, sialidosis type II and mucopolysaccharidosis. Severe liver dysfunction in the neonate can occur in galactosaemia, hereditary fructose intolerance, tyrosinaemia, mitochondrial fat oxidation defects, CDG type Ia and type Ib, $\alpha_1$-antitrypsin deficiency, mevalonic aciduria. Additionally in infancy and pre-school years besides defects seen in neonatal period ketogenesis defects, PC deficiency, urea cycle defects, cholesteryl-ester storage disease, lysosomal diseases and some others may be diagnosed.

The authors present two children with inborn errors of metabolism connected with liver failure. The first patient is a 7-year-old-girl with CPT-1 (carnitine palmitoyl-transferase-1 deficiency) with two attacks of fasting hypoketotic coma and severe abnormalities in liver function tests (such as significant increase in serum transaminases, hyperamonemia, hyperbilirubinemia) coupled with increased markers of muscle damage. The next patient is a 3-year-old-boy suffering from type A Niemann-Pick disease. The boy had been suffering from failure to thrive, hepatosplenomegaly with liver failure and rapidly progressive neurodegenerative course that had led to death at the age of 3 years.
Complement activation is not involved in the pathogenesis of alcohol-induced liver damage in rats

Harri A. Järveläinen1,3, Igor Bykov2, Antti Väkevä1, Seppo Meri1 and Kai O. Lindros2
1Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital, Finland; 2Alcohol Research Center, National Public Health Institute, Helsinki, Finland and 3Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, FIN-00014 University of Helsinki, Finland

Among inflammatory mediator systems that could contribute to the development of alcoholic liver disease (ALD) the complement system has received little attention. We were recently the first to demonstrate the activation of complement components in livers of chronically alcohol-treated animals (Järveläinen et. al., Clin Immunol. 105, 57-63; 2002). In the present study the involvement of the complement system in the pathogenesis of experimental ALD was investigated in C6-deficient PVG rats and corresponding C6+/PVG controls.

After continuous exposure by liquid diet for 6 weeks the deposition of all complement components examined (C1, C3, C8 and C9) was significantly increased by ethanol in C6+/PVG rats. However, these increases were not seen in ethanol-treated C6-deficient rats. Ethanol treatment caused a marked liver damage, such as microvesicular/ macrovacuolar steatosis and focal inflammation. Unexpectedly, in the absence of C6 many indicators of the ethanol-induced liver damage were even increased when compared to PVGc+ rats. These include serum liver enzymes (ALT), serum TNF-α and liver/body weight ratio.

Our results show that deficiency of the C6 component of the terminal C pathways does not alleviate the development of experimental alcohol-induced liver injury and the associated inflammatory cytokine response. This suggests that the activated complement system play no or only a small role in mechanisms of early alcoholic liver disease in rats.
Evaluation of the efficacy of pegylated interferon alfa-2a vs. standard interferon alfa-2a in the treatment of chronic hepatitis C genotype 4 in Egypt

Kabil S.M., M.D., Yousef S.M, M.D., Asaal A., M.D., Gad M.A., M.D., Elkady M.S., M.D, Moawad G., M.D.
Department of Hepatology, Gastroenterologyand Infectiology, Benha Faculty of Medicine, University of Zagazig, and Department of Clinical Pathology Aim Shams University, Cairo, Egypt

Chronic hepatitis C is a major health problem worldwide and elsewhere, it is increasing in number in Egypt. The lack of effective satisfactory therapeutic regim has prompted this trial that aimed to evaluate the efficacy of PEG IFN-alfa-2a either alone or in combination with ribavirin vs. IFN-alfa-2a and ribavirin in the treatment of CHC.

The cohort consisted of 40 patients 39 were genotype 4 with histologically proven CHC with detectable HCV RNA in their sera, they were 29 M, 11 F, with mean age 46.4, were stratified into three groups:

**G1:** 20 patients received PEG IFN-alfa-2a (180 µg) either alone or in combination with ribavirin, they were further divided into:
- G1A: 3 patients received PEG IFN once weekly SC for 48 weeks.
- G1B: 9 patients received PEG IFN once weekly SC for 24 weeks + ribavirin 800 mg/d orally.
- G1C: 8 patients received PEG IFN once weekly SC for 48 weeks + ribavirin 800 mg/d orally.

**G2:** 10 patients received IFN-alfa-2a (3 MU) SC once weekly SC for 24 weeks + ribavirin 800 mg/d orally.

**G3:** 10 patients received supportive treatment and serve as a control group.

They were followed up clinically and laboratory, HCV RNA was estimated after 12 and 24 weeks after treatment.

At presentation SVR was significatly higher in patients treated with PEG IFN + ribavirin for 48 weeks (62.5%) than in patients treated with PEG IFN + ribavirin for 24 weeks (44.4%) however, PEG IFN alone (33.3%) or IFN + ribavirin for 24 weeks (20%). It's therefore concluded that in genotype 4 once weekly PEG IFN either alone or in combination with ribavirin is safe and well tolerated.

**Correspondence:**

E-mail: mostafaelkady@hotmail.com
Markedly altered expression of tight junction proteins between primary human hepatocellular carcinomas and metastatic liver tumors

Andras Kiss, Csilla Paska, Erika Orban, Szijarto A., Kupcsulik P., and Zsuzsa Schaff
2nd Institute of Pathology, Semmelweis Medical University, Budapest, Hungary, 1st Department of Surgery, Semmelweis Medical University, Budapest, Hungary

Background: Tight junction proteins as claudins, occludin and JAM or peripheral zonula occludens (ZO) proteins are widely implicated in carcinogenesis. Claudins (1-24) have been recently identified as integral proteins of tight junction strands. Claudin-4 has not been found in normal hepatocytes or bile duct epithelium.

Aim: The objective was to characterize the expression of claudin 1-4, 7, occludin, JAM 1-2 and ZO 1-3 in human HCCs and liver metastases.

Material and methods: 19 human HCC, 12 colon metastasis samples and nontumorous livers were examined by real-time RT-PCR and immunohistochemistry.

Results: Claudin-4 in HCCs was found downregulated 18-folds and 35-folds compared to normal livers and metastases, respectively. Western blot data confirm minimal expression of claudin-4 in HCC, however, immnohistochemistry detected the presence of claudin-4 on bile ducts. Metastatic tumors showed strong claudin-3 and 4 immunolabeling. Claudin 3 mRNA expression was 9-fold higher in metastases compared to HCC. Claudin 1, nevertheless, showed 4-fold higher expression in HCCs. Claudin-2 revealed 15-fold downregulation in comparison to normal liver, however, there was no significant difference between HCCs and metastases. ZO-2 and JAM-2 RNA expression was 4- and 3-fold decreased in HCCs compared to the surrounding tissue.

Conclusion: Taken together, pronounced downregulation of claudin 3 and 4 and higher expression of claudin 1 might differentiate primary hepatocellular carcinoma from metastatic colorectal tumors. The decreased expression of claudin-2, JAM-1, and ZO-2 was found significant between HCCs and surrounding liver.

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Distinguishing features of cholestasis in pregnancy

J. Kondrackiene, L. Kupcinskas
Department of Gastroenterology, Kaunas Medical University Clinic, Kaunas, Lithuania

Background: Pregnant women may be affected by intrahepatic cholestasis of pregnancy (ICP), intercurrent cholestatic diseases or some of these are exacerbated by the underlying pregnant state. Making the correct diagnosis is very important, as failure to do can result in mortality for the fetus.

Aim: To define most common biochemical abnormalities, evaluate the efficacy of ursodeoxycholic acid (UDCA) in comparison to cholestyramine.

Patients and methods: Serum liver test and bile acids were investigated in 84 patients with ICP. Inclusion criteria were: pruritus and elevation of alanine aminotransferase (ALT) > 45 U/L, aspartate aminotransferase (AST) > 40 U/L, fasting serum bile acids > 10 μmol/L. Exclusion criteria: chronic liver disease, viral hepatitis, skin or allergic diseases, symptomatic cholelithiasis. Patients randomly received UDCA 8-10 mg/kg/d or cholestyramine 8 g/d for 14 days.

Results: Recurrence of ICP was in 25%, cholelithiasis in 18% cases. The most common biochemical signs were: elevated ALT (median 198.5 IU/L, range 14.0-702.0) and AST (136.6 IU/L; 28.0-547.0) in 85%, fasting bile acids (43.8 μmol/l; 5.6-214) in 73%, AP (381.5; 156.0-900.0) in 70%, γ-GT (25.8 IU/L; 8.0-91.0) in 11%, bilirubin in (15.5 mmol/l; 5.0-93.3) in 13% patients. Cholic acid was predominant (49%) in the composition of bile acids (22.8 μmol/l; 1.0-101.6). UDCA, but not cholestyramine significantly reduced pruritus, serum aminotransferases, bile acids.

Conclusions: 1. The most sensitive biochemical parameters were elevated transaminases and bile acids. 2. Cholic acid is predominant in the bile acid pattern. 3. γ-GT is rarely (~ 10%) elevated. 4. UDCA is effective and safe in ICP.
The effect of the combined IFN-α and ribavirin treatment on serum IL-10 levels in patients with chronic hepatitis C

V. Kupcová1, Z. Zelinková1, L. Turecký2, S. Weiszová3
1,3rd Department of Internal Medicine, Dérer’s Hospital, Comenius University, Bratislava
2Institut of Chemistry, Biochemistry and Clinical Biochemistry, Medical Faculty, Comenius University, Bratislava, Slovakia
3Slovak Health Care University, Bratislava, Slovakia

Hepatitis C virus (HCV) infection represents an important public health problem. Cytokines play an important role in viral clearance in chronic hepatitis C (CHC). Several studies have suggested that a specific cytokine profile is related to chronic evolution of HCV infection. This profile is characterised by predominant Th1 cytokine production, intrahepatic (Bertoletti et al. 1997) as well as peripheral (Cacciarelli et al. 1996). On the other hand, the Th2 cytokine intrahepatic production has been found to be decreased (Dharancy et al. 2000, Bertoletti et al. 1997). Interleukin 10 (IL-10) is a Th2 type cytokine that has immunoregulatory properties. The major biological effect of IL-10 is inhibition of proinflammatory cytokines synthesis in monocytes (De Waal Malefyt et al. 1991).

The aim of currently used treatment of chronic HCV infection, administration of a recombinant cytokine - interferon α (IFN-α) and an antiviral nucleosid analogue - ribavirin, is to induce changes in individual immune response that would lead to virus elimination. Interestingly, IFN-α has been shown to induce IL-10 production in human peripheral blood mononuclear cells (Aman et al. 1996). Therefore, we were interested in assessment of the effect of the combined IFN-α and ribavirin treatment on serum IL-10 levels in patients with CHC. At the same time, the relationship of the IL-10 pattern to short virological response to the treatment was studied.

Patients and Methods

The study was conceived as a prospective clinical trial and its procedures were in accordance with the Helsinki Declaration of 1975. An informed consent was obtained from each participant.

Fourteen patients (5 female, 9 male) with CHC were included in the study. Mean age of patients was 41 years, ranging from 21 to 62. Inclusion criteria were positive serum HCV-ribonucleic acid (HCV-RNA) and abnormal alanine aminotransferase (ALT) values for six months and more. Liver biopsy was performed in 12 patients, showing mild to moderate activity in 8 patients and severe in 2 patients (two samples were not representative), in two cases the biopsy was contraindicated. All patients were treated during a period of one year with IFN-α subcutaneous injections of 3 MU three times per week and 10.6 mg per kg of body weight of ribavirin orally. Therapeutic response was determined as disappearance of serum HCV-RNA by week 24 of the treatment. Control group represented eight healthy blood donors (6 female and 2 men), mean age 31 years (ranging from 28 to 39 years), with normal ALT values, anti-HCV antibody and
hepatitis B virus surface antigen (HBsAg) negativity, with no clinical and laboratory signs of allergic or inflammatory condition.

Serum HCV-RNA was assessed qualitatively by polymerase chain reaction (PCR) in serum samples obtained before treatment and in week 24.

Serum IL-10 was measured by a commercially available kit Quantikine® (R & D Systems) that employs the quantitative sandwich enzyme immunonassay technique with microplate coated with murine monoclonal antibody against IL-10. The minimum detectable dose of IL-10 with this kit is typically less than 3.9 pg/mL. Blood samples for serum IL-10 analysis were obtained from each patient before the treatment (Sample 1), in weeks 4 to 8 of the treatment (Sample 2) and in weeks 20 to 28 of the treatment (Sample 3). All patients and controls serum specimens, were stored at -20ºC until they were assayed in the same run.

Nonparametric statistical analysis were applied. Median and ranges are given. Data were tested by Wilcoxon’s test. A significance limit of 0.05 was used.

**Results**

Good therapeutic response determined by disappearance of serum HCV-RNA by week 24 was observed in 8 from 14 patients (57%). The whole patient group median of serum IL-10 levels obtained before treatment (Sample 1) was 2.32 pg/ml with a minimum of 1.55 and maximum 3.3 pg/ml. In the control group IL-10 levels ranged from 1.59 to 2.5 pg/ml with a median of 2.2 pg/ml and were not significantly different in comparison with the patient group.

According to the therapeutic response, patients were divided into two groups: responders (R) with disappearance of HCV-RNA in week 24 (8 patients) and a non-responders (NR) with persistent serum HCV-RNA in week 24 (6 patients). Responders serum IL-10 levels before treatment differed from the control group (p = 0.046) with a median of 2.36 pg/ml (range from 1.99 to 2.92 pg/ml). In non-responders no difference was observed in comparison with the control group, serum IL-10 levels ranged from 1.55 pg/ml to 3.3 pg/ml with a median of 2.18 pg/ml (Fig. 1).

During the treatment, a different pattern of changes in serum IL-10 was noticed in each group of patients. In comparison with levels observed before treatment (Sample 1), responders presented a significant decrease (p < 0.05) of serum IL-10 to 2.07 pg/ml (1.88-2.75 pg/ml) in weeks 4 to 8 (Sample 2). This decline was also present in weeks 20 to 28 (Sample 3), with a median of 2.18 pg/ml (1.95-2.96 pg/ml), differing significantly from values obtained before treatment (p < 0.05) (Table 1). The comparison of responders IL-10 levels with the control group showed raised values before treatment (Sample 1) (p = 0.046), during the treatment (Sample 2 and 3) no differences were observed (Fig. 2). In non-responders group a significant increase of serum IL-10 to 2.75 pg/ml (1.8-3.53 pg/ml) was observed in weeks 4 to 8 (Sample 2) compared to Sample 1 (p < 0.05). IL-10 levels obtained in Sample 2 in this group were also significantly increased when compared with the control group (p < 0.05) (Fig. 2). Increased IL-10 levels in non-responders were still present in weeks 20 to 28 (Sample 3), with a median of 2.44 pg/ml (1.84-3.48 pg/ml), this being significantly increased in comparison with Sample 1 but not differing from control group (Table 1, Fig. 2).
**Figure 1:** Serum IL-10 levels obtained before treatment in comparison with control group (R - responders, NR - non-responders, C - healthy controls)

**Figure 2:** Changes in serum IL-10 levels in chronic hepatitis C patients responding and non-responding to IFN-α and ribavirin treatment compared with control group (R - responders, NR - non-responders, C - healthy controls)
Table 1: Changes in serum IL-10 levels in chronic hepatitis C patients responding and non-responding to IFN-α and ribavirin therapy (* p < 0.05)

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<tr>
<td>RESPONDERS</td>
<td>Sample 1</td>
<td>2.36 (1.99-2.92)</td>
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<td>Sample 2</td>
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<td>Sample 3</td>
<td>2.18 (1.95-2.96)</td>
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<td>NON-RESPONDERS</td>
<td>Sample 1</td>
<td>2.18 (1.55-3.3)</td>
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<td></td>
<td>Sample 2</td>
<td>2.75 (1.8-3.53)</td>
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<td></td>
<td>Sample 3</td>
<td>2.44 (1.84-3.48)</td>
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Discussion

Good therapeutic response was related to a decrease in serum IL-10 during the treatment while in non-responders group an increase was observed. Furthermore, responders showed a tendency to increased pretreatment IL-10 levels. In contrast, the non-responders group presented a significant augmentation of IL-10 in comparison to healthy controls in the second month of the treatment.

Recent studies have showed that IFN-α treatment influenced both, proinflammatory Th1 cytokines (Itoh et al. 1995), as well as Th2 cytokines production (Cacciarelli et al. 1996). However, the relationship of these changes to therapeutic outcome and IFN-α mechanism of action still remains unclear. Despite of lack of concordant results in this field, it seems that a good response to IFN-α treatment might be related to a suppression of proinflammatory cytokines production (Itoh et al. 1995). New insights into IFN-α mechanisms of action pointing out its immunoregulatory and anti-inflammatory effects (Tilg 1997) may provide a biological basis for these clinical findings.

Our observation suggest that the impact of IFN-α and ribavirine therapy on the immunoregulatory cytokine IL-10 is also an important factor for viral clearance. This is in accordance with other finding of decreased IL-10 and IL-4 production (Cacciarelli et al. 1996) relationship to good therapeutic response. One can speculate that the decrease in serum IL-10 accompanies the decline in Th1 cytokine production that has been found to be related to good virological response (Itoh et al. 1995). However, IFN-α has been found to stimulate IL-10 production in activated CD4+ T cells and monocytes (Aman et al. 1996). The decrease of systemic IL-10 levels that was observed in our responding patients may then be explained by an additional effect of ribavirine, as it has been suggested previously (Cramp et al. 2000). Interestingly, our finding of increased serum IL-10 during IFN-α and ribavirine treatment in non-responders, together with above mentioned observations of decreased IL-10 production in responders might suggest that IL-10 immunoregulatory effect is not a decisive factor for viral clearance in hepatitis C.

IL-10 is an immunoregulatory cytokine and is produced by a variety of cells (Napoli et al. 1996). Its decreased intrahepatic mRNA expression has been found in patients with chronic hepatitis C (Napoli et al. 1996, Dharancy et al. 2000). Our patients pretreatment systemic IL-10 levels did not differ from healthy controls, only in responders group we noticed a trend for elevated serum IL-10 before treatment. This may suggest that there is
a difference between intrahepatic and systemic host immune response and the systemic response pattern might be important for therapeutically induced viral clearance.

Based on these results, it can be concluded that a pattern of systemic IL-10 response to IFN-α and ribavirin treatment may be related to short virological response in patients with chronic hepatitis C. The present study results also suggest that serum IL-10 assessment might be used as a response predicting marker in management of patients with chronic hepatitis C treated with IFN-α and ribavirin.
Assessment of iron overload in patients with hereditary hemochromatosis

V. Kupcová¹, S. Durínová¹, M. Szántová¹, L. Turecký², L. Kovács³, J. Zlocha³, V. Belan⁴, 3rd Dept. of Medicine, Dérer’s Hospital¹, Institute of Medical Chemistry and Biochemistry², 2nd Department of Pediatrics, Medical School of Comenius University Department of Nuclear Magnetic Resonance⁴, Bratislava, Slovakia

The liver plays a central role in iron metabolism. It is one of the body’s major storage organs for iron and plasma iron transport protein, transferrin. This is synthesized by the liver. Hereditary hemochromatosis is an autosomal recessive intestinal iron absorption leading to parenchymal iron overload with subsequent tissue damage. The liver, heart, pancreas, joints and pitutary are the main organs affected. Increased iron absorption leads to excess iron accumulation in the liver, where iron concentrations up to 30 to 50 times normal can be seen. In a group of patients with hemochromatosis serum iron, total iron-binding capacity, transferrin, ferritin, transferrin saturation and liver biopsy for histologic interpretation (with iron stains) was investigated. Magnetic resonance (MR) was performed to assess the amount of iron in the liver tissue and to compare it with other investigations (biochemical, hematological, histological, genetical analysis). In all patients the result of MR investigation correlated well with serum ferritin. One patient is followed up for many years and for the last 4 years was treated by phlebotomy. Repeated MR imaging of the liver with assessment of the amount of iron in the liver tissue revealed the decreasing amount of iron during 5-years therapy. Not only reduction of iron in the liver confirmed by MR could be seen, but continually improvement of liver function tests and better clinical state could be seen as well. The patient is now in a good shape. MR imaging with assessing of the amount of iron in patients with hemochromatosis is a helpful investigation not only for confirmation of diagnosis, but also for the follow up of the patient and assessment of therapeutic effect during the treatment as well.
Investigation of interleukin 10 and assessment of hepatic fibrosis in patients with chronic viral hepatitis treated by interferon

V. Kupcová¹, M. Valková¹, Z. Zelinková¹, L. Turecký², S. Weiszová³, E. Jahnová³
3rd Department of Internal Medicine¹, Dérer’s Hospital, Institute of Chemistry, Biochemistry and Clinical Biochemistry², Medical School, Comenius University³ Institute of Preventive and Clinical Medicine, Bratislava, Slovakia

Cytokines are soluble mediators that control many critical interactions among cells of the immune system. Cytokines play an important role in viral clearance in chronic viral hepatitis. Interleukin 10 (IL-10) is a Th2 type cytokine that has immunoregulatory properties. The major biological effect of IL-10 is inhibition of proinflammatory cytokines synthesis in monocytes. IFN-α has been shown to induce IL-10 production in human peripheral blood mononuclear cells. Therefore, we were interested in assessment of the effect of the IFN-α treatment on serum IL-10 levels in patients with chronic viral hepatitis.

Hepatic fibrosis is a reversible accumulation of extracellular matrix in response to chronic injury. We have examined serum hyaluronic acid (HA) concentration and serum IL 10 in a group of patients with histologically verified chronic viral hepatitis B (VHB) and C (VHC) treated 48 weeks by interferon α (IFN-α) and healthy blood donors. Serum IL-10 was measured by a commercially available kit Quantikine® (R & D Systems), the level of HA was estimated with an enzyme-linked binding protein assay (hyaluronic acid “Chugai”). Serum HA level before the treatment correlated with the extent of liver fibrosis (r = 0.87, p < 0.001). We observed statistically significant decrease in HA in all patients (good responders and non-responders as well), after the finishing of the treatment by IFN-α. All patients with VHB and good responders in the VHC group had significantly higher pre-treatment IL-10 levels, when compared to controls. During the treatment, a constant decrease in IL-10 was observed in VHB good responders subgroup, reaching the significant difference only in month 6. In VHC patients in the good responders subgroup a significant decrease in IL-10 levels was observed in month 1, while an increase was observed in non-responders subgroup.

Conclusion: Serum HA measurement is a good and clinically useful non-invasive marker of liver fibrosis. It could be therefore used for monitoring of the stage of fibrosis as a measurement of response to antifibrotic therapy. IL 10 might be useful in the follow up of patients with VHB and VHC treated with IFN-α.

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Macroenzymes of gamma-glutamyltransferase in serum of patients with primary biliary cirrhosis

V. Kupcová, E. Uhlíková, M. Szántová, T. Hanisková, L. Turecký
Medical School, Comenius University, Bratislava, Slovakia

Serum gamma-glutamyltransferase (GMT) is prevalently of hepatic origin and its activity has long been known to be increased in most patients with hepatobiliary diseases. One of the relatively rarer causes of elevated blood plasma enzyme activities are macroenzymes. Biochemically, macroenzymes can be classified into two groups: macroenzymes type 1 and macroenzymes type 2. Macrenzyme type 1 is formed by association of plasma enzyme with specific immunoglobulins. Most cases of this type of macroenzyme were found in patients with autoimmune diseases. The group of macroenzymes type 2 consists of non-immunoglobulin-bound macroenzymes. One of mechanisms for the formation of macroenzymes type 2 is binding of solubilized enzymes to the hydrophobic lipoprotein carrier proteins in circulation.

Aim of the study: To investigate the presence of macroenzyme type 1 of GMT and lipoprotein bound GMT in our patients with primary biliary cirrhosis.

Patients and methods: 25 patients with primary biliary cirrhosis were studied. Macrenzyme type 1 was estimated after precipitation of immunocomplexes with polyethylene glycol 6000 and lipoprotein bound GMT was determined after precipitation of LDL + VLDL with sodium phosphotungstate and MgCl₂. The difference between total serum GMT activity and activity after lipoprotein precipitation corresponds to GMT complexed to LDL + VLDL.

Results: There were in none of our patients detected GMT macroenzyme type 1. In a group of healthy subjects we found only in few individuals non marked activity of GMT complexed with LDL + VLDL which not even in one case did not exceed limit 20 U/l. The activity of GMT complexed with LDL + VLDL higher than 30 U/l we found in 39% of patients with chronic hepatitis and liver cirrhosis other than primary biliary cirrhosis. In the group of patients with primary biliary cirrhosis, the activity of GMT complexed to LDL + VLDL higher than 30 U/l was found in 72% of patients. According to our results, the fraction of GMT complexed with LDL + VLDL is significantly higher in patients with primary biliary cirrhosis than in patients with other hepatobiliary disease.

Correspondence:
Ladislav Turecký
Institute of Biochemistry and Clinical Biochemistry
Comenius University Medical School
Sasinkova 2
SLO-81108 Bratislava
Slovakia
Investigation of advanced glycation end products in liver cirrhosis before and after liver transplantation

V. Kupcová¹, K. Šebeková², R. Schinzel³, A. Heidland⁴
3rd Department of Medicine, Medical School, Comenius University¹, Institute of Preventive and Clinical Medicine², Bratislava, Slovakia, Institute of Physiological Chemistry II, Biocenter³ and Department of Internal Medicine⁴, University Würzburg, Germany

Advanced glycation end products (AGEs) are a heterogeneous group of molecules, formed in vivo both by non-oxidative and oxidative reactions of sugars and their adducts to proteins and lipids. They accumulate in tissues and circulation during aging, as well as in diabetes and chronic renal failure. Beside the kidney, also the liver seems to be involved in the removal of AGEs. Two binding proteins of AGEs, OST-48 and 80K-H, have been demonstrated in membranes of the liver. According to this data, an accumulation of AGEs is conceivable in advanced liver disease as a consequence of decreased liver mass. Therefore we have examined the plasma concentration of AGEs in patients with different forms of liver cirrhosis and following liver transplantation. Plasma AGE levels (fluorescent AGEs - AGE-Fl, and N⁵-carboxymethyllysine - CML) were determined in 51 patients with liver cirrhosis (Ci) and 19 healthy controls. 5 patients were observed for 36 months after liver transplantation. In cirrhotic patients markedly elevated concentrations of AGEs were revealed (AGE-Fl: control: 0.3 ± 0.01 x 10⁵ AU, Ci: 1.06 ± 0.06 x 10⁵ AU, p < 0.01; CML: control: 431.7 ± 16.3 ng/ml, Ci: 647.6 ± 258.5, p < 0.01). CML levels correlated with the severity of liver disease, as determined by Child-Pugh score (r = 0.663, p < 0.001), albumin level (r = 0.704, p < 0.001) and monoethylglycinexylide test (r = 0.852, p < 0.01). Reduced renal function contributed to the rise of CML in proportion to the degree of renal impairment. Liver transplantation resulted in about 50% decline of CML levels within 3 months, while impairment of renal function persisted, underlying the central role of the liver for AGE removal.

Conclusion: Accumulation of AGEs results from impaired hepatic removal, itself caused by decreased effective liver mass. In the light of recent knowledge on toxicity of AGEs their accumulation could be of pathophysiological relevance.
The effect of probiotics on gut flora and level of endotoxin in cirrhotic patients - Preliminary results of double blind randomized study

J. Lata, V. Pribramska, J. Jurankova¹, M. Senkyrik
Department of Gastroenterology and Microbiology¹, University Hospital Brno, Czech Republic

The aim of study: Infections are very frequent in patients with liver cirrhosis and play role in fatal complications (variceal bleeding, spontaneous bacterial peritonitis). Increase of bacterial translocation in cirrhotic patients due to the increased permeability of intestinal mucous membrane barrier seems to be important. Probiotics are living microorganisms of human origin that have favourable effects on these mechanisms. The aim of our study was to determine the effect of probiotics to gut flora and level of endotoxin in cirrhotics.

Patients and methods: Fifteen patients with liver cirrhosis Child-Pugh B and C were randomized to the treatment with *E. coli Nissle* (Mutaflor®) (eight patients) or placebo (seven patients) for 42 days. Microbiologic quantitative analysis of stool and level of endotoxin were investigated before and after the treatment.

Results: Dysmikrobia in the stool was found in 12 patients before the treatment. In the probiotic group, in 6 patients was restored normal stool flora and the endotoxin level significantly decreased in 4 patients. There were no changes in the placebo group.

Conclusion: Probiotics seems to be effective in the restoration of physiological flora in the gut in cirrhotics and can decrease the level of endotoxin. As an infection plays important role in etiology of many complications of liver cirrhosis, decrease of possibility of infection can be important.

The research was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic (NK 7366-3).
Possible prevention of gallstone formation: Effect of *Raphacol bile-granule* on lipid peroxidation characteristics in diabetic patients

A. Lugasi\textsuperscript{1}, S. Kassai-Farkas\textsuperscript{2}, T. Horváth\textsuperscript{2}, A. Blázovics\textsuperscript{3}

\textsuperscript{1}“Fodor József” National Center of Public Health, National Institute of Food Hygiene and Nutrition, Budapest, \textsuperscript{2}St. Borbála County Hospital, Tatabánya, \textsuperscript{3}2nd Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Recently significant association of gallstone diseases with diabetes has been emphasized. Natural antioxidants can play a crucial role in prevention of diseases where free radicals are involved. Dietary compounds such as ascorbic acid, tocopherol and carotenoids beyond their antioxidant properties are to be considered as basic regulatory agents in gene expression. Black radish root has been suggested to use against abdominal discomfort, indigestion, inflation and for increase of bile secretion, and according to recent observations this complex plant material exhibits marked antioxidant activity \textit{in vitro} and also in animal experiments. *Raphacol bile-granule* contains squeezed juice from 60 g fresh black radish root and 0.25 g aetherolum foeniculi completed with granulum simplex to 100 g. The parameters characterising the lipid peroxidation in diabetic patients were studied during a 6 months intervention period while subjects consumed regularly 0.6 g/day *Raphacol bile-granule*. Patients' average daily intakes of the antioxidants from black radish juice were 18 μg ascorbic acid, 0.07 μg β-carotene, 1.1 μg tocopherol, 92 μg polyphenols including 1.76 μg kaempferol and 0.23 μg quercetin. Patients were under regular medical control and received adequate treatment of diabetes. After 6 months the lipid peroxidation characteristics improved and majority of the patients reported the elimination of the abdominal inconveniences in both diabetic groups. Results suggest that regular consumption of *Raphacol bile-granule* may help to prevent the gallstone formation via inhibition of lipid peroxidation; it can improve the antioxidant status in diabetic patients, and result in a more comfortable life for them, as well.

The study was supported by the Hungarian Scientific Research Foundation, Hungary (OTKA T 043537).
Latent encephalopathy in patients with compensated liver cirrhosis

P. Migdalski, J. Juszczyk
Department of Infectious Diseases, Karol Marcinkowski University of Medical Sciences in Poznan, Poland

**Materials and methods:** In 50 healthy volunteers (25 males, 25 females, mean age $x = 42 \pm SD 14$ years) in standard and equal conditions psychomotor test was done. The time in each test was measured and the normal range value was determined as $x \pm 2 SD$.

The same tests were used in 50 patients with compensated liver cirrhosis of different etiology (37 males, 13 females, mean age $x = 46 \pm SD 8$ years). The time needed for each test was compared in both groups (patients and control group).

**Results:**

<table>
<thead>
<tr>
<th>Test</th>
<th>normal range</th>
<th>values above the normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numeric</td>
<td>below 2 min.</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>Alphanumeric</td>
<td>below 3 min.</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Labyrinth</td>
<td>below 1 min.</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Pentagram</td>
<td>below 1 min.</td>
<td>12 (24%)</td>
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</table>

In group of patients with compensated liver cirrhosis:

- 34 cases (68%) were professionally active, in 15 patients (46%) psychomotoric disorders were observed.
- 36 cases (72%) drove a car (professionally 4).
- 8 patients did work demanded special psychophysical conditions, in 4 cases the time was prolonged for at least one test.

**Conclusions:**

1. Latent encephalopathy is a relatively common symptom in patients with compensated liver cirrhosis.
2. Psychomotoric tests should be standard diagnostic examination in patients with liver cirrhosis, especially in those who are professionally active.
Chemokines expressed in the gut and inflamed liver regulate lymphocyte adhesion to MAdCAM-1

Alice E Miles, Bertus Eksteen, Patricia F Lalor, David H Adams
Liver Research Laboratories, Institute for Biomedical Research, University of Birmingham, Birmingham, B15 2TT, UK

Mucosal cell adhesion molecule-1 (MAdCAM-1) is constitutively expressed in the intestinal tract and associated lymphoid tissue and plays an important role in the trafficking of lymphocytes to the gut. IBD may be associated with inflammation at extra-intestinal sites including the liver; primary sclerosing cholangitis (PSC) shows a strong association with IBD, although it often follows a course independent of gut inflammation. MAdCAM-1 expression has been detected in PSC liver and it has been proposed that aberrant hepatic MAdCAM-1 may allow recruitment of gut-derived memory lymphocytes to the liver, leading to inflammation.

Lymphocyte adhesion to MAdCAM-1 requires a chemokine signal to trigger integrin activation and we have shown previously that the gut-associated chemokines CCL25 and CCL28 can also be detected in PSC liver, as can CCL21, a lymph node chemokine known to activate lymphocyte adhesion to MAdCAM-1. In order to determine which chemokines are important for activating binding to MAdCAM-1 we developed a flow-based adhesion assay to investigate lymphocyte adhesion to immobilised MAdCAM-1 under conditions of shear stress. Immobilised CCL21, CCL25 and CCL28 were all able to convert rolling adhesion of normal PBLs to integrin-mediated arrest on MAdCAM-1. This effect was G-protein dependent as it was inhibited by pertussis toxin. When co-immobilised with VCAM-1 these chemokines had no effect, suggesting they may act specifically in stimulating adhesion to MAdCAM-1. Studies using liver infiltrating lymphocytes from PSC patients have demonstrated that CCL25 can activate adhesion of these lymphocytes to MAdCAM-1 providing further mechanistic evidence for the link between liver and gut inflammation.
The changes of the liver's biochemical parameters after partial resections of the gastrointestinal tract

Jacek Paszkowski, Tomasz Banasiewicz, Ryszard Marciniak, Jacek Szmeja, Hanna Perz, Michal Drews  
Department of General, Gastroenterological and Endocrinological Surgery, K. Marcinkowski University of the Medical Sciences, Poznan, Poland

The aim of our study was to evaluate the metabolic function of the liver after different types of gastrectomy or partial colectomy. We compared biochemical parameters of blood (albumin, bilirubin levels and alanine amino-transferase (ALAT), asparagine amino-transferase (ASPART) and alkaline phosphatase activity) before and after surgery.

Our study group consisted of 75 patients. 30 patients were operated on for gastric cancer - without metastases, 30 for colon cancer without metastases and 15 operated on for inguinal hernia - control group.

In the first two groups we observed laboratory features of transitional metabolic dysfunction of the liver. We found increased activity of ALAT, ASPAT, alkaline phosphatase and increased level of bilirubin. Albumin levels (low before surgery) were decreased. Main changes were observed during first fewdays after operation. Biochemical abnormalities were small. The level of examined parameters 3 weeks after surgery were nearly the same as the preoperative stage. In the control group, no changes were found.

Gastrectomy or colectomy, affecting digestive and absorptive processes, disturbs liver metabolism. Monitoring of liver function and treatment of biochemical abnormalities should be an important component of patient follow-up after partial resection of the gastrointestinal tract. It is very important especially for patients, who had liver diseases prior to operation.
The effect of ras inhibition on the proliferation, activation and matrix metalloproteases expression of hepatic stellate cells

Shimon Reif, Dan Bar-Zohar, Isabel Zvibel, Zamir Halpern, Ran Oren
Pediatric Gastroenterology, Unit Liver Unit and Gastroenterology institute Tel-Aviv Sourasky Medical Center, Israel

Background/Aims: We have previously shown that in vivo inhibition of ras by its antagonist trans-farnesylsalicylic acid (FTS) prevents and reverses liver fibrosis in a rat model. In order to further explore the mechanism of the anti-fibrotic effect of FTS, we have studied the in vitro effects of ras inhibition in hepatic stellate cells cultures, including effect of ras inhibition on cell proliferation and activation and on the expression of matrix metalloproteases (MMPs).

Methods: Immortalized hepatic stellate cell line HSC-T6 was used in this study. Inhibition of PDGF-induced cell proliferation by FTS was assessed by cell counting. Signal transduction, α-smooth muscle actin (α-SMA) and MMP-13 expression were determined by Western blots. Activity of MMP-2 and MMP-9 was assessed by zymograms. Expression of tissue-inhibitor of metalloprotease-2 (TIMP-2) was determined by RT-PCR.

Results: We have found that the IC50 of FTS that inhibited PDGF-induced proliferation was 15 μM. This inhibition was accompanied by decreased activation of mitogen-activated protein kinase erk1/erk2 in the presence of PDGF and FTS. There was no effect of FTS on the expression of both cell-cycle stimulator cyclin D1 and cell-cycle inhibitor p21. Moreover, FTS, by itself or in combination with PDGF, induced a three- to five fold increase in the number of apoptotic stellate cells. We observed a two- to three fold increment in the activity of secreted MMP-2, induced by FTS alone, or in combination with PDGF or TGF-β. In parallel, the expression of TIMP-2 was augmented by FTS with either PDGF or TGF-β in a similar manner. There was no effect of FTS on MMP-13 expression. Finally, FTS had an inhibitory effect on the differentiation and activation of stellate cells, demonstrated by decreased expression of α-SMA.

Conclusions: The amelioration of liver fibrosis by FTS may be explained by its ability to inhibit hepatic stellate cells proliferation and activation and to induce MMP-2 activity.
Increased tacrolimus levels due to grapefruit juice ingestion

Y. Rosenbach, I. Zahavi, G. Dinari
Division of Gastroenterology and Nutrition, Schneider Children's Medical Center of Israel, Petach-Tikva, Israel

Tacrolimus (FK-506) blood levels need to be closely monitored in post transplant patients. Tacrolimus level depends on its intestinal absorption and metabolism, which is mainly hepatic. A one-year-old girl had liver transplantation due to fulminant hepatic failure of unknown cause. Tacrolimus and prednisolone were initiated. Its levels were kept around 10 ng/ml. Ten months after uncomplicated follow-up, blood levels were unexpectedly elevated to 37 ng/ml on two occasions and confirmed by another laboratory. Several days before this event, the child started to consume large amounts of grapefruit juice. Avoidance of grapefruit juice reduced tacrolimus blood levels to normal. Various drugs, such as cyclosporine and grapefruit flavanoids are metabolized through cytochrome P-450 in the liver. Grapefruit ingestion elevates cyclosporine and other drugs blood levels. Tacrolimus is also metabolizes through the cytochrome P-450 system, but there is no documentation about the effect of grapefruit juice on Tacrolimus levels. It is proposed by us that its elevated blood level in our patient in conjunction with grapefruit consumption is due to the effect on the cytochrome P-450 system. Patients, treated with tacrolimus should be aware of the possible effect of grapefruit on tacrolimus blood levels.
The effect of magnesium on the redox homeostasis in hyperlipidemia

Klára Szentmihályi¹, Ibolya Kocsis², Erika Rapavi², Gabriella Taba¹,², Judit Fodor¹, László Váli², Anna Blázovics²
¹Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
²Semmelweis University, Budapest, Hungary

Hypomagnesemia is one of the common features of liver diseases as fatty liver, alcoholic and other cirrhosis. Decrease of the magnesium level in serum, erythrocyte, lymphocyte, liver tissues, heart and skeleton muscle as well as bone is known. Since magnesium plays a key role in the synthesis of several proteins and enzymes, the maintaining of the antioxidant system as well, our purpose was to investigate the effect of a magnesium compound/complex in vivo. Magnesium polygalacturonate complex of natural origin was applied and the redox homeostasis was examined in hyperlipidemic rats.

Male Wistar rats (n = 40; 150-200 g bw) were divided into four groups (control, control + Mg-treated, hyperlipidemic, hyperlipidemic + Mg-treated). After 9 days routine laboratory parameters and redox homeostasis (plasma: scavenger capacity, H-donating ability and reducing power; erythrocyte: scavenger capacity; liver: scavenger capacity, H-donating ability, reducing power and diene-conjugates) were measured.

Routine laboratory parameters showed that by the effect of magnesium therapy the ALP, creatinine, cholesterol and amylase activity decreased compared to the rats with fatty liver. The scavenger capacities of examined tissues increased and the concentrations of diene-conjugates decreased significantly in treated rats.

On the basis of the results the magnesium therapy improved the function of antioxidant system in vivo in hyperlipidemia.

The study was supported by NKFP 1/047/2001.
Plasma fibrosis markers alpha-2-macroglobulin and hyaluronate in patients with primary biliary cirrhosis

L. Turecký, V. Kupcová, S. Ilavská, M. Szántová, E. Jahnová, E. Uhlíková
Medical School, Comenius University, Bratislava, Slovakia

Primary biliary cirrhosis (PBC) is a chronic, progressive biliary autoimmune disease of the liver, exhibiting immunologic abnormalities and leading to inflammatory destruction of small- and medium-sized bile ducts. In the advanced stage the biliary inflammation has progressed to fibrosis leading finally to cirrhosis and liver failure. Liver biopsy is the gold standard for assessing fibrosis, but liver biopsy is invasive and prone to complications. The use of biochemical parameters as fibrosis markers could substantially reduce the number of biopsies. Recently, several studies reported relative very good correlation of alpha-2-macroglobulin (AMG) to the activity of liver fibrosis.

Aim of the study: To investigate the levels of AMG, potential fibrogenesis marker, in patients with PBC. To compare the levels of AMG with levels of hyaluronic acid which is generally accepted as marker of liver fibrogenesis.

Patients and methods: The concentrations of hyaluronate and AMG were determined in 24 patients with PBC (diagnoses were histologically verified). The level of hyaluronic acid was estimated with an enzyme-linked binding protein assay and AMG was estimated immunochemically by electroimmunoassay using monospecific antisera.

Results: The levels of both parameters were increased in patients with PBC. When patients were divided according to their clinical status, the levels of hyaluronic acid and AMG were significantly higher in the group of patients with decompensated progressive disease than in the group with stabilized patients showing no progression of disease. There was also significant correlation between the levels of AMG and hyaluronic acid concentrations.

Conclusion: According to the results of our study we can conclude that the estimation of AMG could be helpful in the diagnosis and monitoring of liver fibrosis also in patients with primary biliary cirrhosis.

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Correspondence:
Prof. Ladislav Turecký, MD, PhD
Institute of Medical Biochemistry and Clinical Biochemistry
Medical School
Comenius University
Sasinkova 4
SLO-81108 Bratislava
Slovakia
Relationship between cholestasis and fibrogenesis in patients with primary biliary cirrhosis

L. Turecký, V. Kupcová, S. Ilavská, M. Szántová, E. Uhlíková
Medical School, Comenius University, Bratislava and Institute of Preventive and Clinical Medicine, Bratislava, Slovakia

Fibrogenesis is currently viewed as a dynamic process strictly related to the extent and duration of parenchymal injury. The precise relationship between cholestasis, in its broad meaning, and liver tissue fibrosis is still poorly defined. Marked and progressive liver tissue fibrosis always follows liver diseases characterized by chronic inflammatory bile duct damage (i.e. primary biliary cirrhosis) or chronic mechanical obstruction of the biliary tree.

Aim of the study: To investigate the relationship between levels of plasma bile acids, which are accepted as sensitive cholestatic marker, and liver fibrogenesis markers (hyaluronic acid and alpha-2-macroglobulin).

Material and methods: The concentrations of total bile acids, hyaluronic acid and AMG were determined in 24 patients with PBC (diagnoses were histologically verified). The level of hyaluronic acid was estimated with an enzyme-linked binding protein assay and AMG was estimated immunochemically by electroimmunoassay using monospecific antisera.

Results: The levels of plasma total bile acids were significantly increased in patients with primary biliary cirrhosis in comparison to controls (77.79 μmol/l vs. 4.23 μmol/l). The patients with progressive, decompensated disease in more advanced stage had higher levels of plasma bile acids than patients with stabilized disease (115.62 μmol/l vs. 21.03 μmol/l). The levels of fibrogenesis markers were also significantly higher in decompensated patients than in patients with compensated disease (hyaluronic acid: 98.79 ng/ml vs. 30.11 ng/ml, alpha-2-macroglobulin: 4583 mg/l vs. 2895 mg/l). The regression analysis showed statistically significant correlation between the levels of total bile acids and fibrogenesis markers (bile acids vs. macroglobulin, r = 0.65, bile acids vs. hyaluronic acid, r = 0.72).

In conclusions, the results of our study showed that cholestasis could be one of the factors which are involved in the pathogenesis of fibrosis in patients with primary biliary cirrhosis.

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Correspondence:
Prof. Ladislav Turecký, MD, PhD
Institute of Medical Biochemistry, Medical School, Comenius University, Sasinkova 2 SLO-81372 Bratislava, Slovakia
Lipoperoxidation activity in patients with primary biliary cirrhosis

E. Uhlíková, V. Kupcová, M. Szántová, I. Vozár, L. Turecký
Institute of Medical Chemistry and Biochemistry and IIIrd Medical Clinic, Medical School, Comenius University, Bratislava, Slovakia

Primary biliary cirrhosis (PBC) is a chronic, progressive biliary autoimmune disease of the liver, exhibiting immunologic abnormalities and leading to inflammatory destruction of small- and medium-sized bile ducts. In the advanced stage the biliary inflammation has progressed to fibrosis leading finally to cirrhosis and liver failure. It is known, that in the pathogenesis of inflammatory destruction of tissues could participate also free reactive radicals. Estimation of plasma conjugated dienes reflects the activity of lipoperoxidation induced by free reactive oxygen radicals.

**Aim of the study:** To estimate the activity of lipoperoxidation in patients with primary biliary cirrhosis and the correlation between the activity of lipoperoxidation and levels of acute phase proteins which reflect the activity of inflammatory process.

**Patients and methods:** The concentrations of conjugated dienes were determined in 19 patients with PBC (diagnoses were histologically verified). The method used for the estimation of conjugated dienes in blood plasma of our patients is based on the extraction and estimation of oxidized unsaturated fatty acids with conjugated bindings at 234 nm. The level of alpha-1-acid glycoprotein, alpha-1-antitrypsin, ceruloplasmin and haptoglobin were determined immunochemically by electroimmunoassay using monospecific antisera.

**Results:** The level of conjugated dienes were significantly increased in patients with primary biliary cirrhosis. There was relatively good correlation between the levels of acute phase proteins and the level of conjugated dienes (correlation coefficients from $r = 0.29$ to $r = 0.49$, $P < 0.05$) in despite of not increased levels of acute phase proteins. The fact, that we didn't found significant increase in the levels of acute phase proteins in our group of patients with primary biliary cirrhosis could be explained by decreased liver proteosynthetic capacity in these patients.

In conclusions, results of our study showed that the activity of lipoperoxidation was increased in our group of patients with primary biliary cirrhosis. The correlation between activity of lipoperoxidation and activity of inflammatory process support the hypothesis about the role of oxidative stress in the pathogenesis of liver damage in patients with primary biliary cirrhosis.

**Correspondence:**
Dr. Eva Uhlíková, PhD
Institute of Medical Biochemistry, Medical School, Comenius University, Sasinkova 2, SLO-81372 Bratislava, Slovakia
Steatosis of the liver as the manifestation of Wilson disease in a 14-year-old boy - Case report

Sabina Wiecek, Urszula Grzybowska-Chlebowczyk, Halina Wos, Maria Szymanska
Department of Pediatrics, Silesian Medical University, Katowice, Poland

INTRODUCTION: Wilson disease is a genetically determined metabolic disease and it is rarely diagnosed in children. A liver damage is non-characteristic and it can manifest itself only in abnormal laboratory tests (increased aminotransferases).

DESCRIPTION OF THE CASE: We present a case of a 14-year-old boy with obesity and hyperlipidemia, who has been diagnosed since he was 10 because of increased levels of aminotransferases. When the patient was 12 years old steatosis of the liver was found (non-alcoholic steatohepatitis was diagnosed); in spite of dietetic and pharmacological treatment normal laboratory tests were not achieved. The boy was referred to our Department of Gastroenterology with a view to the further diagnostics; the tests revealed: decreased level of ceruloplasmin in blood serum and increased copper excretion in urine, which indicated Wilson disease. Pharmacological treatment was administered in this patient. The boy is an ambulatory patient in Gastroenterology Outpatient Clinic.

CONCLUSIONS: In this study we intended to pay attention to a non-characteristic clinical picture of Wilson disease in children and validity of broadening of diagnostics towards metabolic disorders in patients with steatosis of the liver (despite of unequivocal features of hyperlipidemia and obesity).
Early diagnosis and treatment of newborns with necrotizing enterocolitis

Ana-Maria Bradeanu, MD, Roxana Dinca, MD, Laura Carasava, MD
Clinical Children's Hospital "Gr. Alexandrescu", Bucharest, Romania

Introduction: Necrotizing enterocolitis (NEC), one of the most serious surgical conditions during neonatal period is characterized by inflammation, necrosis and even perforation of the gut.

Objective: The purpose of this study is to investigate the role of both early diagnosis and appropriate treatment in order to decrease the significant morbidity and mortality associated with NEC.

Methods and results: Between January 2000-September 2004 we studied 53 cases of NEC representing 7.41% from 715 admissions in our NICU. The patients were diagnosed and staged according to the modified Bell criteria. Frequent clinical reevaluation, laboratory data and sequential abdominal X-rays monitored the evolution of the disease. Medical supportive management was begun immediately as the diagnosis was suspected. All the patients received a double or a triple association of systemic antibiotics for at least 10-14 days, but the hemocultures were positive only in 15 cases. A number of 19 patients (35.8%) from the studied group required surgical interventions due to intestinal perforations and peritonitis. All survivors received enteral nutrition, with a semielemental, lactose free formula, after 5-12 days of peripheral TPN.

Conclusions: Early recognition of this life threatening condition, rapid and adequate management of infants with NEC is critical to minimize the progression of the disease. Thus our yearly mortality decreased from 80% in 2000 (when we grounded our NICU) at 10% in 2004.
Effects of ursodeoxycholic acid (UCDA) on endogenous ethanol and intestinal bacterial flora in rats fed methionine-choline deficient diet (MCDD)

Institute of Biochemistry, National Academy of Sciences, Grodno, Belarus
Department of Microbiology, Grodno State Medical University, Belarus
Dr. Falk Pharma GmbH, Freiburg, Germany

Many authors believe that administration of UDCA as a preparation for treatment of non-alcoholic steatohepatitis (NASH) may have a beneficial effect. However, this problem is still under study and the data from clinical trials are controversial. The mechanisms of a hepatoprotective effect of UDCA in rat experimental NASH are still unclear and some authors attribute them to antioxidant properties of UDCA (Buko et al., 2004) or to increases in a hepatic blood flow (Kurihara et al., 1993). The aim of this study was to evaluate the effects of UDCA on the endogenous ethanol contents and intestinal microflora in rats with NASH induced by a MCDD feeding.

Male Wistar rats (180-200 g) were fed MCDD during 10 weeks. Rats from four MCDD-fed groups were daily administered with UDCA (10, 20, 40 and 80 mg/kg b.w., i.g.) during the last two weeks on the experiment. One of the MCDD-fed groups was decapitated after 6 weeks of the feeding for base line values before starting UDCA.

Severity of liver damage was evaluated morphologically and biochemically. Endogenous ethanol was measured in blood, intestinal contents and mucose by gas-chromatography. Bacterial spectra of both intestinal contents and mucose were evaluated by routine microbiological methods.

The morphometrical data suggest that the relative square of liver sudanophily (% to total slide square) significantly increased only after 10 weeks of the MCDD feeding. The treatment with UDCA dose-dependently decreased liver damage, but only UDCA (80 mg/kg) diminished this parameter with statistical significance. The activities of serum marker enzymes, ALAT and ASAT, gradually elevated to the same extent during the feeding with MCDD and decreased in all the UDCA-treated groups.

The blood endogenous ethanol content increased in MCDD-fed rats from nearly 5-fold after 6 weeks to about 22-fold after 10 weeks of the MCDD feeding. The treatment with UDCA (40 and 80 mg/kg) decreased significantly this parameter. Similarly to blood, the endogenous ethanol content in intestinal mucose increased during the NASH development but the decreasing effect of UDCA on this parameter was maximal for the dose of 20 mg/kg and minimal for the dose of 40 mg/kg. Two doses of UDCA, 10 mg/kg and 80 mg/kg, lowered this parameter to the same extent. The endogenous ethanol concentration in intestinal contents was similar in all the investigated groups.
We observed formation of intestinal dysbacteriosis in MCDD-fed rats which was manifested as changes in the main bacterial group content and bacterial groups ratio \((E.\ coli; Lactobacterium,\ spp;\ Bifidobacterium;\ Clostridium,\ etc.)\). The intensity of this dysbiotic phenomenon was dependent on the duration of the MCDD feeding. The normalizing effect of UDCA on the intestinal biocenosis, which we observed in some individual groups of bacteria was dependent on the UDCA dose, whereas the dose 40 mg/kg most effectively changed the quantitative composition of both aerobic and anaerobic components of the intestinal biocenosis.

The results obtained give grounds to develop further studies on intestinally derived ethanol which may contribute to the pathogenesis of NASH. We assume that one of the mechanisms of hepatoprotection by UDCA in NASH may be realized via a decrease of endogenous ethanol concentration.
Dyspeptic syndrome - Presentation symptoms and endoscopic findings

Liana Chicea, Aurel Sbârcea
Internal Medicine Department, Academic Hospital Sibiu, Romania

We examined the endoscopic findings in a sample of 47 hospitalized patients (16 women and 31 men), admitted for an ulcer-like dyspepsia, among which 26% were on medication. We found that some have acid risk factors as following: 42% were smokers, 26% had significant caffeine intake, 16% declared alcohol intake. Helicobacter pylori was present in 17%. Endoscopic diagnosis was duodenal ulcer in less than a half of the cases (46%), GERD in 25%, and gastric ulcer, gastritis for the remaining cases.

It was no difference in the incidence of duodenal, gastric ulcer, GERD or gastritis in patients with or without any of the considered risk factors.

Presentation symptoms were dominated by heartburn in esophagitis (100%) and GERD (72%), nausea and vomiting (59%) or heartburn (50%) in duodenal ulcer, sour taste (66%) in gastric ulcer, bloating (66%) in gastritis.

Distension of the stomach indicates duodenal ulcer (RR = 2.43, p < 0.05, 95% CI), a bitter taste was suggestive for gastric ulcer (RR = 3.88, p < 0.05, 95% CI), and pyrosis for GERD (RR = 3.6, p < 0.05, 95% CI), while nausea, bloating, poor appetite could not be associated to specific endoscopic findings.
Diagnosing bowel diseases in admitted patients - Are imaging methods rationally used?

Liana Chicea, Oana Purcar
Internal Medicine Department, Academic Hospital Sibiu, Romania

Bowel diseases are frequent in medical practice. We assessed the role of contrast X-ray and colonoscopy in establishing the diagnostic and we looked into the concordance of the clinical diagnosis with the imagistic (final) diagnosis.

Analyzing the medical records of the 371 patients that were admitted in the gastroenterology, internal medicine and surgery departments, we found that 75% underwent one or both of the diagnostic methods mentioned. Colonoscopy was ordered in one third of the patients, most frequently by gastroenterologists, X-rays was ordered mainly by surgeons, in one quarter of all patients. The final diagnosis was concordant with the initial one in less than 50% of the cases.

A more careful approach in taking patient's history, in clinical evaluation might decrease the cost to benefit ratio in using imagistic diagnostic methods.
Duodenal biopsy and celiac disease

Dana Damian, Cristina Pojoga, O. Pascu, M. Grigorescu
Third Medical Clinic, Cluj-Napoca, Romania

We studied 175 patients (55 males and 120 females) that underwent duodenal biopsies during a period of time of 7 months (December 2003-May 2004).

The biopsies were performed for the following reasons: diarrhea (124 cases), anemia (24), malabsorbive syndrome (3), reducing of the duodenal folds observed during upper endoscopy (9), inflammation of the duodenal mucosa observed during upper endoscopy (9), one parent with celiac disease (1), diarrhea and loss of the duodenal folds (1), presence of a pancreatic or choledocian tumour (4).

11 of the 175 patients (6.28%) were diagnosed with celiac disease, the histological diagnosis being completed by the finding of the antigliadine antibodies. All the patients were female, aged between 27 and 38 (mean age 30.09). 10 patients (90.90%) had presented to the doctor because of a diarrheic syndrome and one of them had also a reducement of the duodenal folds at the upper endoscopy. One of the patients with diarrhea had subocclusive episodes, with no lymphoma found at the examination of the intestine and colon.

One of the patients (9.09%) had no diarrhea, malabsorption or anemia, but the endoscopist observed the reduction of the duodenal folds during the upper endoscopy, deciding to take a biopsy (the gastroscopy being performed for other reasons).

The results show that the diagnosis of celiac disease is not infrequent in the adult age, the biopsy of the duodenum being necessary in all the cases of diarrhea.
Manipulation of the enteric environment with functional foods containing probiotics may offer protection against bowel disease

Corneluta Fira-Mladinescu¹, O. Fira-Mladinescu², Danina Muntean², Violetta Vacariu³, Smaranda R. Gotia⁴, Brigitha Vlaicu¹, Sorina Doroftei¹
Departments of ¹Hygiene, ²Pathophysiology, ³Internal Medicine, and ⁴Physiology, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania

Background: Because the intestinal microflora plays an important role in the development of inflammatory bowel disease (IBD), there is currently some interest in the manipulation of the human gut ecosystem. The aims of the present study were: (i) to investigate the modality by which functional foods with probiotics, such as Lactobacillus and Bifidobacterium yogurt (Activia®), may influence the intestinal function and (ii) to establish the role of fermented milk derivates in the IBD ethiopathogenesis.

Methods: The assessment of intestinal motility was performed in a randomized group of 246 normal individuals by the means of stool frequency recording, while 43 patients with recently diagnosed IBD and 84 control subjects matched for sex, age and the socio-economic status were studied in a case-control design. Fermented milk products ingestion was evaluated by food frequency questionnaires.

Results: The average daily intake of Activia® was of 148 ± 84 g in individuals reporting one stool daily vs. 65 ± 42 g in those with abnormal bowel motility. Decreased intakes of fermented milk derivates were positive associated (RR = 3.57, p < 0.01) with an abnormal intestinal function. A high intake of these functional foods was negative associated with IBD (OR = 0.35, p < 0.01) in the case control study.

Conclusion: Manipulation of enteric microecology by using functional foods containing probiotics may be useful in ensuring a normal bowel function, being useful in preventing intestinal diseases such as IBD.

Correspondence:
Corneluta Fira-Mladinescu
"Victor Babes" University of Medicine and Pharmacy
Department of Hygiene
Z. Steaua bl. 24, sc. A, ap. 4
RO-300446 Timisoara
Romania
Tel/Fax: +40 256 220 479
E-mail: mladinescu@umft.ro
Use of probiotics in childhood postinfectious irritable bowel syndrome

M. Georgieva-Shakola, A. Atanassova*, V. Tzaneva, R. Pancheva
Department of Pediatrics, Department of Gastroenterology, University Hospital "St.Marine", Varna, Bulgaria

BACKGROUND: Irritable bowel syndrome (IBS) might develop after bacterial gastroenteritis in adults and in children. Probiotics are useful in the treatment of several gastrointestinal diseases in childhood, including infectious diarrhea, antibiotic diarrhea etc. Little is known regarding probiotics and IBS after intestinal bacterial infection in children.

AIM: To determine the efficacy of probiotics - Lactobacilli (L. acidophilus and L. bulgaricus) + non-pathogenic yeast Saccharomyces boulardii (SB) in the treatment of postinfectious IBS in children.

MATERIALS & METHODS: In 2003-2004, 52 children (6 months-6 years) (31 boys and 21 girls) were diagnosed as postinfectious IBS (Escherichia coli, Shigella, Salmonella, Klebsiella) - 1: confirmed by stool culture from the microbiology laboratory without prior IBS; 2: IBS was diagnosed via Rome II criteria 4 weeks after the end of the treatment of bactreial gastroenteritis. They were diveded in two groups - A: 25 patients were treated with dietary recommendations (increased dietary fat, diminished carbohydrates); B: 27 patients + additional treatment with probiotics - Lactobacilli bulgarici 0.8 million live cells and 16 milliards latent cells/daily + Lactobacilli acidophili 2 billions live cells/daily + SB 0.5 mg orally for 2-4 months. The patients were followed for a period of 6 months.

RESULTS: At the 4th week the group with probiotic therapy has shown a significant attenuation of clinical features - loss of abdominal pain (19/27 contra 12/25), normalization of stool frequency (20/27-13/25), normalization of stool consistency (22/27-13/25), loss of mucorrhea (19/27-15/25) In the group A there were no relapses of IBS, in the group B 2 children were with 3 relapses. Probiotics were well tolerated, safe, without adverse reactions.

CONCLUSIONS: This study suggests that Lactobacilli and SB can be effective in the treatment of postinfectious IBS in childhood. The real efficacy of these probiotics - their doses, duration of application needs further evaluation. They are an attractive additional treatment option for IBS.

Correspondence:

Dr. Miglena Georgieva-Shakola
Department of Pediatric Endocrinology & Gastroenterology
University Hospital "St. Marina", 1, "Ch. Smirnenski" Str., BG-9000 Varna, Bulgaria
Predicting signs of necrotizing enterocolitis

E. Goldstein
Division of Pediatric Gastroenterology, Schneider Medical Center for Children, Petach-Tikva, Israel

Necrotizing enterocolitis (NEC) is one of the most frequent gastrointestinal diseases in low birth weight premature neonates. To identify the predicting signs of NEC, clinical course and outcome were examined. Weight gain and liver function tests were evaluated in 15 NEC and control patients. Weight gain and clinical data of NEC patients are presented in the table.

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*Exitus

Elevated weight gain among NEC patients was the most significant difference between the two groups. Average weight gain was 7% compared to 1% in control patients and it is explained by edema of the intestinal wall and fluids accumulated in the abdomen leading to low blood pressure and renal output. No significant difference was observed in regarding to liver function tests. Weight gain and not liver function tests is important in predicting the clinical course of NEC patients.
Role of substance P and corticotropin-releasing hormone in regulation of barrier function in rat ileum during acute stress

Åsa Velin Keita1, Rakel Eriksson1, Ann-Charlott Ericson2 and Johan D Söderholm1
Department of Biomedicine and Surgery, Faculty of Health Sciences, University Hospital, Linköping, Sweden, 1Division of Surgery, 2Division of Cell Biology

Background & Aim: Stress affects the course of inflammatory bowel disease, but the mechanisms are unknown. The earliest observable sign of Crohn's disease is microscopic erosions over the follicle-associated epithelium (FAE), covering Peyer's patches. The FAE provides a route of entry for microorganisms, and animal studies have shown an increased uptake in FAE after stress. Our aim was to evaluate how stress in combination with corticotropin-releasing hormone (CRH) and Substance P (SP) influence mucosal permeability.

Methods: Twenty-five rats were injected intraperitoneally with CRH receptor antagonist, NK1 receptor antagonist or sodium chloride and submitted to acute water avoidance stress. Ileal segments of villus epithelium (VE) and FAE were dissected and permeability was studied in flat sheets of imucosa mounted in Ussing chambers by measuring fluxes of the protein antigen horseradish peroxidase (HRP), 51Cr-EDTA and Escherichia coli K-12.

Results: Stress substantially increased bacterial and antigen uptake in both VE and FAE, however the increase was higher in FAE. The bacterial increase was totally abolished by CRH receptor antagonist, whereas the NK1 receptor antagonist had no effect. The stress-induced increase in HRP permeability was significantly blocked by both antagonists. In contrast, 51Cr-EDTA permeability was significantly decreased by NK, receptor antagonist while unchanged by CRH receptor antagonist.

Conclusions: Stress affects the FAE barrier to antigen and bacteria with mechanisms involving CRH and SP. Our results indicate that CRH is involved in uptake of both antigen and bacteria while SP affects only antigenic transport. These findings may have implications for the pathogenesis of Crohn's disease.
Antigliadin antibodies in the diagnosis of celiac disease in children

Lesanu G, Becheanu C, Stanescu M, Stanescu D
Department of Pediatric Gastroenterology Emergency Children Hospital Bucharest, Romania

The aim of the study is to analyze the antigliadin antibodies (AGA) as a method to detect the patients for small bowel biopsy in order to diagnose celiac disease (CD)

Patients and methods: We performed a retrospective study to analyze the cases admitted in Emergency Children Hospital who were screened for serum immunoglobulin A (Ig A)-AGA by means of an enzyme-linked immunosorbent assay (ELISA) method. We investigated 1809 symptomatic children (age: 8 months-18 years) admitted for: chronic diarrhea, mild gastrointestinal symptoms less suggestive for CD, extradigestive manifestations (syderopenic anemia, failure to thrive, short stature).

Results: We found 968 patients with chronic diarrhea screened for AGA; 69 (7.1%) were positive, 63 of them underwent intestinal biopsy and 57 had villous atrophy.

Among 185 children admitted for atypical gastrointestinal symptoms, 24 were positive for AGA, 22 underwent biopsy and 12 (5.4%) had villous atrophy. There were 264 children investigated for short stature, 13 were positive, 12 accepted biopsy, 10 had villous atrophy (3.7%). We found 227 children admitted for failure to thrive, 15 (6.6%) were positive and had villous atrophy. Among 137 children with syderopenic anemia, 10 were positive for AGA, 9 underwent biopsy, 7 (5.1%) had villous atrophy.

Conclusions: Determination of AGA proved to be a useful tool in identifying CD in children with chronic diarrhea, or less suggestive clinical manifestations. We found a high incidence of CD in symptomatic children 1/18.
The symptomatology of irritable bowel syndrome at patients with ulcerative colitis in remission

Simona Popa, Amelia Genunche
University of Medicine and Pharmacy Craiova, Romania

The symptoms of irritable bowel syndrome (IBS) - abdominal pains, alleviated by defecation, distended abdomen, and meteorism - at patients with ulcerative colitis (UC) in remission can be determined by other associated disorders - depressive mood, migraine, palpitation, problems in micturition (irritable bladder), and lower abdominal pains of gynecological origin.

Aim: Evaluation of patients with UC in remission and the correlation of clinical symptomatology with IBS.

Material: The study was made on a group of 47 patients with UC in remission evaluated clinical, biological and endoscopic.

Results: The repartition of our group indicated the prevalence of women (63.8%), with age between 30-50 years.
The symptomatic patients (87.2%) had in their past history: depressive mood (46.4%), migraine (12.5%), palpitation (7.3%), problems in micturition (19.5%), lower abdominal pains of gynecological origin (12%), symptoms frequently met in IBS. Comparing the group of symptomatic patients with that of the asymptomatic patients, no correlations were found between the presence of symptomatology and the duration and extension of the disease.

Conclusions: The symptoms of IBS occur of the most patients with UC in remission, predominating at female sex with psychiatric diseases. There is no correlation between the presence of symptoms and the duration and extension of the disease.
Induction of particle trancytosis across human intestinal epithelial cells (Caco-2) after exposure to Yersinia pseudotuberculosis

Ida Schoultz¹, Eva Ragnarsson², Elisabet Gullberg², Karl-Eric Magnusson³, Johan D Söderholm¹ and Per Artursson²
¹Department of Surgery and Clinical Research Center, University Hospital, Linköping, ²Department of Pharmacy, Uppsala University, Uppsala, ³Division of Medical Microbiology, Department of Health and Environment, Linköping University, Sweden

Purpose: To evaluate and quantify the transport of particles across human intestinal epithelial cells (Caco-2) after exposure to Yersinia pseudotuberculosis expressing or lacking invasin A (InvA).

Methods: Caco-2 cells were grown on permeable filters and exposed on the apical side for YadA deficient Yersinia pseudotuberculosis with or without the adhesin invasin A (Inv+ and Inv-). Uninfected monolayers were used as control. After exposure the transport of fluorescently labeled polystyrene particles (200 and 500 nm) from the apical to the basolateral side of the cell monolayer was quantified using flow cytometry. Transepithelial resistance was measured to monitor the integrity of the cell monolayers. Particle transport was also evaluated by confocal scanning microscopy.

Results: Caco-2 cells exposed for Inv+ bacteria showed a size and concentration dependent increase in transport of model particles across the intact monolayers. The transport of particles across control and Inv- exposed monolayers were not increased. Confocal images showed that the Inv+ bacteria were co-localized with particles on the cell surface and also particles internalized into cells.

Conclusions: The results show that the transport capacity of Caco-2 cells was affected after exposure to Inv+ Yersinia pseudotuberculosis.
Dyslipidemia in solid organ transplantation in children

R. Shapiro¹, A. Silbermintz¹, E. Mor², N. Bar-Nathan², Z. Ben-Ari³, G. Dinari¹
Institute of Gastroenterology and Nutrition, Schneider Children’s Medical Center of
Israel¹, Department of Transplantation² and Institute of Hepatology³ Rabin Medical
Center, Petah Tikva, Israel

Dyslipidemia is common after organ transplantation in adults. It is attributed mainly to
immunosuppression. There is very little information regarding dyslipidemia in
transplanted children.

Aims: To study the prevalence and causes of dyslipidemia in transplanted children.

Patients and methods: Post transplantation serum lipids were assessed in 30 liver
transplanted children (aged 38 ± 4.9 ms) and 7 children (aged 46.4 ± 16.3 ms) who
underwent combined liver and kidney transplantation. There were 22 boys and 15 girls.

Results: All patients with combined organ transplantation were on steroids. There was
significant increase in cholesterol (169.4 ± 8.4 vs 140 ± 4.8, mean ± SEM, p = 0.014)
and in LDL levels (91.3 ± 8.0 vs 69.4 ± 4.1, p = 0.036) in patients who underwent liver
and kidney transplantation, compared to those who received only a liver transplant.
Similarly, there was significant increase in cholesterol (157.5 ± 7.3 vs 131.6 ± 4.9, mean
± SEM, p = 0.006) and in LDL levels (81.9 ± 7.1 vs 64.1 ± 4.3, p = 0.042) in patients on
tacrolimus (n = 17) compared to those on tacrolimus and steroids (n = 15).

Conclusions: Although lipid levels remained within normal limits, total cholesterol and
LDL levels were elevated in combined organ transplantation and in those on steroids.
Children with liver and kidney transplantation, especially those on steroids, may be at
risk for developing dyslipidemia and its consequences, and should be carefully followed
up for this complication.
Table beet extract during ischaemia-reperfusion

László Váli, Mária Takács-Hájos, Hedvig Fébel, Éva Stefanovits-Bányai, Ibolya Kocsis, Gabriella Taba, Klára Szentmihályi, János Fehér, Anna Blázovics
Semmelweis University, Budapest, Hungary

Bioactive agents have beneficial antioxidant properties. Our aim was to determine the activity of bioactive substances of beetroot in the model of ischaemic-reperfusion injury of the rat liver.

Free radical intensity (FRI), diene-conjugates (DC), H-donating ability (HDA), reducing power (RP), Randox-TAS, -GSHPx and -SOD activities were detected. Metal ions of the liver were measured with ICP-AS, and fatty acids with Shimadzu GC.

Wistar rats (n = 32) were divided into control and beetroot-fed groups. During nembutal narcosis hepatic ischaemia was induced for 45 min, which was followed by 15 min of reperfusion. Animals were treated with beetroot liophylized extract (2 g/bw kg/day; betanin content: 64.55 mg/100 g, polyphenol content: 72.0 mg/ml) for ten days.

Liophylized extract of table beet reduced the FRI of plasma significantly. The other antioxidant parameters of plasma were increased in treated rats, and RP was significant. Concentrations of DC were reduced significantly in liver, due to the feeding, and HDA, as well as RP was increased in treated rats. Extract increased the values of TAS and SOD in the liver. Plasma lipid concentrations were lowered in treated animals. Based on these data it can be concluded, that beetroot can protect the liver from the oxidative damage caused by ischaemia-reperfusion, but opposite change was detected of FRI of the duodenum during ischaemia-reperfusion due to beetroot-feeding. HDA and RP of the gut were decreased in the beetroot-fed group as well.

Supporting: ETT-002/2003
The damage of the liver in infants and children with cystic fibrosis

Wos H.¹, Grzybowska-Chlebowczyk U.¹, Szymanska M.¹, Pawlik J.², Pogorzelski A.², Zebrak J.²
¹Department of Pediatrics, Silesian Medical University, Katowice, Poland
²Clinic of Bronchology and Cystic Fibrosis, Institute of Tuberculosis and Lung Diseases, Pediatric Division, Rabka, Poland

Damage of the liver in the course of cystic fibrosis has been known for a long time, but only during last few years this pathology has drawn the attention of a bigger group of doctors.

THE AIM OF THE STUDY is to present the rate of clinical and biochemical symptoms occurrence which accompany damage of the liver in children with cystic fibrosis.

MATERIALS AND METHODS: The study included children with diagnosed cystic fibrosis; 14 infants aged 2-9 months (mean age 5.5/12) and 34 children aged 2-18 years (mean age 7.5) diagnosed and treated in Department of Pediatrics at Silesian Medical University and Clinic of Bronchology and Cystic Fibrosis in Rabka. The liver diseases were defined on the strength of clinical criteria (hepatomegaly), biochemical (increase of liver enzymes activity) bilirubin and in 2 patients depicting examination was performed (usg of abdominal cavity, in elected cases CT of abdominal cavity).

RESULTS: In the group of infants pathology of the liver was observed in 11 out of 14 examined patients (71.5%). Liver damage in 2 patients manifested itself in the form of hepatomegaly and cholestatic jaundice, in 1 patient Reye-like syndrome was diagnosed; in the rest of patients only the increase of aminotransferases was detected. In the group of older children damage of the liver was observed in almost half of the examined (48.2%), mainly as an asymptomatic hypertransaminasemia, in 2 patients with enlargement of the liver in usg examination. Additionally, in 5 patients steatosis of the liver was found, in 1 patient-cholecystolithiasis. In this group of children there was 1 who had intense pathology of the liver observed since infancy with cirrhosis of the liver complicated by oesophageal varices bleeding, now after a successful transplantation of the liver. In 2 children (an infant 9/12 and a boy aged 9) CT revealed accompanying hypoplasia of the pancreas.

CONCLUSION: With regard to the high rate of liver damage occurrence in patients with cystic fibrosis we should periodically evaluate liver function using biochemical examination and usg, because clinical symptoms occur only in very pathologically advanced cases.
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