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P300
Correlation of age and serum dipeptidyl peptidase IV (DPP IV/CD26) activity in patients with inflammatory bowel diseases

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Crohn's disease (CD) and ulcerative colitis (UC) are the best known forms of inflammatory bowel diseases (IBD). Their etiology is still unclear, but recent studies indicated the involvement of the immune system in the pathogenesis. Dipeptidyl peptidase IV (DPP IV/CD26) is a membrane-bound multifunctional glycoprotein, acting as a proteolytic molecule, receptor, binding and costimulatory molecule. The proteolytic cleavage of the membrane bound DPP IV results in a soluble form that migrates in the plasma. It has previously been shown that the DPP IV/CD26 could play a significant role in the pathophysiology of IBD. The aim of this study was to determine the influence of patient's age on the serum DPP IV/CD26 activity in patients affected with IBD. The research was performed on 93 patients, divided in 2 groups: 31 young patients (mean age 13.8±1.7 years, 24 with CD and 7 with UC) and 62 adult patients (age 42.7±14.4 years, 38 with CD and 24 with UC). The control group included 111 healthy blood donors: 46 children (age 13.8±2.8 years) and 65 adults (age 41.6±12.1 years). Serum DPP IV/CD26 activities in both young and adult patients with IBD were found to be statistically significantly decreased when compared to their healthy controls. Values correlated inversely with the disease severity for both CD and UC. When comparing serum DPP IV/CD26 activities between young and adult patients with IBD, but even between young and adult healthy controls, it was noticed that serum DPP IV/CD26 activity decreases statistically significantly with age. The results of this study show that serum DPP IV/CD26 could be useful as an available, non-invasive marker in the diagnosis of the disease activity. This research also shows that age-related standard values should be undoubtedly established in clinical laboratory practice because of the age-dependent decrease in serum DPP IV/CD26 activity.

P301
Tumor necrosis factor-alpha gene polymorphism in Russian patients with ulcerative colitis

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Tumor necrosis factor (TNF)-alpha play a key role in inflammatory response in Crohn's disease and ulcerative colitis (UC). The aim of our study was to determine the effect of two single nucleotide polymorphisms (SNPs) of the TNF-alpha gene, TNF -308 G/A and TNF -238 G/A, their influence on inflammatory activity and the clinical manifestations in Russian patients with UC.

Methods: The distribution of -308 and -238 TNF-alpha genotypes was analyzed in 74 patients with UC and 116 healthy controls.

Results: The genotype and allelic frequency of TNF-alpha -308 in patients with UC were 18.9% and 12.2%, respectively, significantly higher than in control population (6.8% and 4.3%, respectively; p < 0.05). Carrying of -308A allele of TNF-a gene increases the UC risk (OR = 3.09). We found that patients with -308G/A genotype had higher level of platelets (p = 0.045) and more frequent arthritids (p = 0.048) in active disease compared with patients with -308G/G genotype. No difference in the G→A substitution at position -238 was observed.

Conclusion: TNF-alpha -308 G/A polymorphism may play a role in UC in Russian patients, who have more intensive inflammatory activity.

P302
Innate immune suppression by trace metals – an unexpected role in the pathogenesis of Crohn’s disease?

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Aim: A deficient innate immune response in the gut and ensuing excessive adaptive immune activation has been implicated in the pathogenesis of Crohn’s disease (CD), but the cause for this inadequate innate immunity has not been determined. The aim of this study was to investigate the possible role of dietary trace metals in the immunopathogenesis of CD.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained from CD patients (n = 17) and healthy controls (n = 13). Cellular proliferation of CD4+ T-cells, and secretion of TNF-alpha and IL-8 by PBMC were assessed after addition of beryllium, aluminum or zirconium, in the presence or absence of LPS or TSST stimulation. Apoptosis of CD4+ T-cells and CD14+ monocytes was similarly examined by AnnexinV/PI assay.

Results: None of the metals stimulated proliferation of CD4+ T-cells, nor elicited cytokine secretion by PBMC. Surprisingly however, beryllium significantly and selectively reduced IL-8 secretion by LPS-activated or TSST1-triggered monocytes (693 ± 667 vs. 409 ± 371 mcg/ml, P = 0.002), an effect that was not observed with the other metals. Importantly, this inhibition of IL-8 production was not mediated by any discernable toxic cell death or apoptosis induction. Moreover, beryllium-induced suppression of IL-8 secretion could be partially reversed by the co-incubation of cells with GM-CSF, a recently proposed agent for the treatment of CD.

Conclusions: These data indicate that beryllium specifically inhibits a key cytokine in the innate immune response to bacterial LPS. This inhibitory effect could possibly undermine the immune-bacterial homeostasis in the gut, leading to excessive immune activation and ensuing chronic inflammation.

P303
Peroxisome proliferator-activated receptor gamma (PPARγ) expression and activation in colonic epithelial cells from patients with ulcerative colitis: a randomized, controlled pilot study of the effect of topical treatment with rosiglitazone enemas

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Background and Aim: The peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor highly expressed in normal human colon, where it attenuates inflammatory responses. Impaired PPARγ expression has been described in colonic epithelial cells in animal models and in patients with active ulcerative colitis (UC), but the functional significance in humans is not well described. The primary aim of this study was to describe the expression pattern and in vitro function of PPARγ in human colonic epithelium. Secondly, we examined the clinical effect of treatment with rosiglitazone enemas (a PPARγ ligand) in a small controlled study in patients with active distal ulcerative colitis.

Methods: Spontaneous and rosiglitazone-induced PPARγ- and adiophillin expression (a PPARγ-activated gene) were measured by RT-PCR in primary short-term cultures of colonic epithelial cells isolated from endoscopic biopsies from controls (n=8) and UC patients (n=8). Fourteen UC patients with active proctitis or proctosigmoiditis were randomised
to treatment with either rosiglitazone (4 mg/100 ml) or mesalazine (1000 mg/100 ml) enemas once daily for 14 days. Disease activity was measured by the Mayo score. Adipophillin expression was measured at inclusion and after 24 hours in isolated colonic epithelial cells from all patients.

**Results:** The median PPARγ expression level was significantly reduced in colonic epithelial cells from inflamed UC mucosa (0.5 AU, range 0.1–0.9 AU) compared to un-inflamed mucosa from the same patient (1.8 AU, range 1.1–6.6 AU) and controls (2.2 AU, range 1.1–2.8 AU) (p < 0.01). PPARγ activity, as measured by the median adipophillin expression level, was also significantly decreased in epithelial cells from active UC patients (11.0 AU, range 2.4–17.2) compared to un-inflamed samples (28.5 AU, range 4.8–38.8 AU) (p < 0.05) Stimulation with rosiglitazone (100 μg/ml) in short term cultures of colonic epithelial cells induced a median 3.1 fold increase in adipophillin expression levels compared to unstimulated cultures (range 1.6–10) (P < 0.02), thus restoring the impaired level of PPARγ activation. Topical treatment with rosiglitazone enemas in patients with UC induced significant clinical improvement after 14 days, similar to that of mesalazine enemas (Table). Interestingly, treatment with rosiglitazone enemas (but not the 5-ASA control) induced a rapid and significant increase in the median adipophillin expression level (2.8 AU, range 1.4–3.9 AU) compared to pretreatment levels (1.5 AU, range 0.6–2.2 AU) (p < 0.05) in epithelial cells.

**Conclusion:** PPARγ expression and activity are impaired in inflamed UC epithelium, and stimulation with a synthetic PPARγ ligand can restore PPARγ activity in vitro. Topical treatment with a PPARγ ligand enema seems to have a beneficial therapeutic effect in patients with active distal UC.

Effect of rosiglitazone and mesalazine enema treatment in UC patients

<table>
<thead>
<tr>
<th>Mayo score, mean±SD</th>
<th>Pre-treatment</th>
<th>After 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosiglitazone</td>
<td>8.7±1.2</td>
<td>4.2±0.9*</td>
</tr>
<tr>
<td>Mesalazine</td>
<td>8.6±1.0</td>
<td>3.4±2.0*</td>
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*p < 0.01 compared to pre-treatment.

P304 Galectin-2 and galectin-4 modulate intestinal wound healing, induce T cell apoptosis and thus ameliorate intestinal inflammation in an experimental model of murine colitis

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**Aim:** Inflammatory bowel diseases are characterised by an uncontrolled immune reaction in common with an impaired T cell apoptosis. The resulting mucosal damage impairs epithelial cell function and its barrier function. Galectins, a family of sugar-binding lectins, are known to influence apoptosis and regulate immune cell homeostasis. However their effect on mucosal restitution and the mucosa associated immune system is still unclear. For this we analysed the effect of galectin-2 and galectin-4, both predominantly expressed in the gastrointestinal tract, on the mucosal immune system.

**Material and Methods:** Cell migration was determined by a well-established wound healing assay with Caco-2. PBT were stimulated with cross-linked anti-CD3/28 mAB and cultured in the presence or absence of galectin-2 or galectin-4. Flow cytometry analysis was used to determine apoptosis (Annexin-V), necrosis (PI) and cell cycle progression (Cyclin B1). Cytokine secretion was determined by CBA analysis. Acute colitis was induced in BALB/c mice by adding 5% DSS to the drinking water. Mice were treated i.p. with either NaCl as control, galectin-2 or galectin-4 (1 mg/kg BW) three times a day. The disease activity and histological inflammation index were quantified by established scores. TUNEL staining was performed to determine mucosal apoptosis.

**Results:** A wound healing assay with epithelial cells showed that galectin-2 and galectin-4 promote epithelial cell restitution, with an increase in cell migration and cell proliferation. Both galectins induced apoptosis of stimulated, but not resting T cells. Determination of the cytokine secretion profile of T cells revealed a decrease in pro-inflammatory cytokines, like TNF-α, IL-6, IL-8 and IL-17, when incubated with galectin-2 or galectin-4. In a model of DSS-induced colitis in mice, galectin-2 and galectin-4 treatment significantly ameliorated clinical and histological signs of colitis compared to controls. Analyses of the cytokine secretion profile showed a lowered secretion of pro-inflammatory cytokines. Determination of apoptosis in mice treated with galectin-2 or galectin-4 showed a significant increase in apoptotic CD3 positive cells in the mucosa.

**Conclusion:** Crohn’s disease is characterized by an uncontrolled T cell proliferation and impaired apoptosis. Thus, our study provided for the first time evidence that galectin-2 and galectin-4 play a central and distinct role in the mucosal immune system. These findings may provide a new approach in the treatment of diseases with an impaired T cell apoptosis, e.g. inflammatory bowel diseases.

P305 The molecular mechanism of action of anti-TNF antibodies in inflammatory bowel diseases

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**Introduction:** The anti-TNF antibodies Infliximab, Adalimumab and Cetolizumab pegol have proven clinical efficacy in the treatment of Crohn’s disease. The role of an apoptosis inducing effect of these substances in their molecular mechanism of action is still unclear. Therefore the aim of this project is the identification of a common mode of action of anti-TNF antibodies in inflammatory bowel diseases (IBD).

**Methods:** Antigen presenting cells and CD4+ T-cells were isolated from peripheral blood mononuclear cells (PBMCs) and lamina propria mononuclear cells (LPMCs) from IBD patients and controls. TNF-receptor 2 (TNFR2) expression rate was assessed via flow cytometry. Immunofluorescence staining of gut cryosections showed a significant increase of mTNF expression in lamina propria APCs compared to CD4+ T-cells in IBD patients. The TNFR2 expression was elevated in gut CD4+ T-cells of IBD patients. Treatment with the anti-TNF antibodies in co-cultivated intestinal CD4+/CD14+ cells from IBD patients resulted in an induction of apoptosis in CD4+ T-cells, while this effect was absent in LPMCs of controls or when these cells were cultured alone. TRAF-2 expression was elevated in cell extracts isolated from intestinal CD4+ T-cells.

**Results:** Histological analysis of gut cryosections showed a significant increase of mTNF expression in lamina propria APCs compared to CD4+ T-cells in IBD patients. The TNFR2 expression was elevated in gut CD4+ T-cells of IBD patients. Treatment with the anti-TNF antibodies in co-cultivated intestinal CD4+/CD14+ cells from IBD patients resulted in an induction of apoptosis in CD4+ T-cells, while this effect was absent in LPMCs of controls or when these cells were cultured alone. TRAF-2 expression was elevated in cell extracts isolated from intestinal T-cells from IBD patients.

**Discussion:** Our data show for the first time, that all clinically effective anti-TNF agents are able to induce in vivo apoptosis in gut CD4+ T-cells when co-cultured with CD4+ T-cells from IBD patients. Augmented mTNF expression in gut APCs and elevated TNFR2 expression in CD4+ T-cells in IBD, indicate that this pathway mediates the resistance of T-cells to apoptosis, contributing to the perpetuation of inflammation. Specific targeting of this pathway by anti-TNF antibodies results in binding to mTNF on CD14+ cells, causing an indirect rather than a direct induction of apoptosis in CD4+ T-cells. These data concerning the mechanism of action of anti-TNF antibodies