100% thickening of the smooth muscle wall. Despite equivalent levels of TK expression in enteric glia in the colon, no cell loss or pathological changes in this organ could be detected. Immunohistochemical studies revealed a selective loss of GFAP-expressing glia from the small intestine. This was accompanied by a moderate degeneration of myenteric neurons. Concommitant administration of GCV and antibiotics prevented bacterial overgrowth but had no effect of ileal pathology. Myeloperoxidase activity, derived from neutrophils, confirmed that the inflammatory response was ileal specific and was unaffected by the antibiotic treatment. However, a role for bacteria in the manifestation of pathology cannot be ruled out. CONCLUSIONS:These findings demonstrate that enteric glia are essential to maintaining the integrity of the small bowel and identify dysfunction of this cell population as an upstream trigger for the induction of inflammation with the gastrointestinal tract.

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Opposite Response Of Melatonin In Experimental Acute And Chronic Colitis In Rats

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Background: Melatonin (MEL), in addition to its hormonal function, has antioxidant and immunoregulatory effects and is protective in some models of oxidative damage and inflammation. Inflammatory bowel diseases (IBD) are multifactorial disorders whose etiology involves mainly the immune system. In the TNBS model, its topic administration results in acute transmural necrosis, which is likely caused by oxidative damage, and during the few days after administration, the chronic inflammation is characterized by immune response under a complex regulation by mononuclear and other cells subsets. Aims: To determine the contribution of MEL to the modulation of the TNBS-induced acute and chronic IBD in rats. Material and method: Animals were treated i.p. with different doses of MEL (0.5, 1, 2 mg/Kg) 24 and 1 h prior to acute colitis induction (intrarectal administration of TNBS, 10 mg in ethanol) as well as 24 and 48 h afterwards. Animals were sacrificed 1 h after the last administration. In chronic colitis (30 mg of TNBS), animals received 1 mg/Kg of MEL, 24 and 1 h prior to TNBS and once daily until the sacrifice, 1 or 3 weeks after. Food intake, body weight or the presence of diarrhea were measured daily. The severity of colonic inflammation was assessed macroscopical and microscopically and by measurement of tissue myeloperoxidase (MPO) concentration. TNF-α release and collagen deposition (OH-proline content) were also analyzed. Results: *Acute colitis* - treatment with the three doses of MEL reduced MPO levels ($p<0.05$) and improved some parameters indicatives of colitis or intestinal lesion (global score MEL 2 mg/Kg, $p<0.05$ vs TNBS reference group). *Chronic colitis* - when MEL was tested for 1 - 3 weeks, the macroscopic but also the microscopic study revealed clear signs of worsening. The ulcer was covered with a necrotic exude over a wide area of granulation tissue and only an incipient epithelization was observed. The MPO activity was elevated in all damaged groups ($p<0.001$ vs sham) without significant differences among treated or untreated animals. Moreover. TNF-α production and signs of fibrosis, with important OH-proline content, were significantly detected in MEL treated animals. Conclusions: Although data from the acute study revealed certain preventive role of MEL in the model of IBD assayed, its chronic administration has a negative repercussion in the healing process. Clear worsening in the late inflammatory response and development of fibrosis with proliferation of smooth muscle cells and fibroblast were observed.

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Processing and Activation of α-Defensins in Mouse Intestinal Paneth Cells

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BACKGROUND: Paneth cells in small intestinal crypts secrete microbicidal α-defensins, termed cryptdins, in response to bacterial infection *ex vivo*. In mice, procryptdins must be activated by matrilysin (MMP-7) to produce functional 3.5 kDa α-defensin peptides, and MMP-7 null mice are defective in clearing enteric infections *in vivo*. AIMS: To determine the intracellular distribution and the processed state of cryptdin precursors in mouse Paneth cells. METHODS: Recombinant mouse procryptdin-1 was purified to homogeneity after expression as maltose-binding protein fusions in *E. coli*. Mouse small intestinal crypts were prepared by dissociation with EDTA, and Paneth cells granule fractions were prepared from crypts by differential centrifugation. Proteins were extracted with 30% acetic acid, and proteins were analyzed electrophoretically and in western blots. Jejunum was fixed, embedded in Unicryl at -20°C, sectioned, and stained with rabbit anti-cryptdin/protein A gold (10 nM) and sheep anti-prosegment/donkey anti-sheep gold (20 nM). RESULTS: Peptide sequencing of MMP-7 digests of recombinant procryptdin-1 identified two cleavage sites: between S43-V44 in the proregion and between S58-L59, the latter site being at the junction of the proregion and the cryptdin-1 peptide amino terminus. Cryptdin-1 and its proregion co-localized to Paneth cell secretory granules, and the prosegment inhibited the bactericidal activity of cryptdins 3 and 4 against *E. coli in vitro*, suggesting a cytoprotective role for the proregion prior to secretion. Analyses of protein extracts from full-thickness small intestine, purified crypts, or Paneth cell subcellular fractions enriched for secretory granules showed that 60-70% of the procryptdin pool is processed intracellularly during granulogenesis. No activated cryptdins were detectable in protein extracts from MMP-7 null mouse small bowel. The apparent level of cryptdin activation was equivalent for samples from germ-free or conventionally-reared mice and was unaffected by the inclusion of protease inhibitors. CONCLUSION: MMP-7 processing of mouse Paneth cell α-defensin precursors is primarily intracellular, providing activated bactericidal cryptdins for release into the crypt lumen for mucosal innate immune function.

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Neutrophil Transmigration In Inflammatory Bowel Disease Is Associated With Altered Expression Of Intercellular Junction Proteins

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BACKGROUND: Disease activity in ulcerative colitis (UC) and Crohn's disease (CD) is characterized by transepithelial migration of neutrophils (PMN) and altered epithelial barrier function. Since epithelial barrier function is primarily regulated by the apical most intercellular junction referred to as the tight junction (TJ) our goal was to analyze the expression of intestinal epithelial intercellular junction proteins in colonic epithelium of patients with active/inactive IBD. MATERIAL AND METHODS: Snap frozen sections and paraffin-embedded sections of colonic mucosa from 23 patients with UC or CD were analyzed by immunoflourescence/confocal microscopy and immunhistochemistry for expression of TJ (occludin, claudin-1, ZO-1, Junction Adhesion Protein) and its subjacent adherens junction (AJ) (E-cadherin, beta-catenin) proteins. Morphologic results were confirmed by western blot of TJ and AJ proteins from mucosal samples. Controls included analysis of colonic mucosa from 18 normals and from patients with other inflammatory conditions such as microscopic colitis (lymphocytic, collagenous) and acute self limited colitis. RESULTS: Analysis of acutely inflamed intestinal tissues from patients with UC revealed a global down-regulation of the key epithelial TJ transmembrane protein occludin. Not only was occludin expression absent in epithelial cells immediately adjacent to transmigrating PMN, there was a diffuse loss of occludin at sites distant from active inflammation. In contrast expression of other TJ and AJ proteins, claudin-1, ZO-1, JAM, E-cadherin and beta-catenin was lost only in epithelial cells immediatly adjacent to transmigrating PMN. Immunoblotting revealed decreased expression of all of these TJ and AJ proteins, however, occludin was down-regulated most dramatically. Analysis of inflammed mucosa from Crohn's disease patients mirrored the results obtained with UC patients. When compared to controls, no down regulation was seen in normal colonic mucosa or mucosa with chronic inactive colitis, lymphocytic and collagenous colitis or infectious colitis. CONCLUSION: In active IBD, PMN transepithelial migration is associated with a diffuse reduction of occludin expression and with loss of other TJ proteins only at sites of activity. We speculate that global down-regulation of epithelial occludin may account for the increased paracellular permeability observed in IBD.

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Dysregulated Production of IEC-Derived Cytokines During Early vs. Established Disease in the SAMP1/Yit Model of Spontaneous Ileitis.

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Background: The intestinal epithelium represents the primary barrier between gut luminal antigens and the mucosal immune system. The aim of this study was to characterize IEC-derived cytokine profiles in early vs. late phases of disease in SAMP1/Yit mice. These mice spontaneously develop chronic ileitis closely resembling human CD with 100% penetrance by 40 weeks of age. A feature of this unique murine model is epithelial alterations, including villous blunting, crypt elongation with increased cellularity, and goblet as well as Paneth cell hyperplasia. These changes in epithelial architecture are observed as early as 4 weeks of age. Methods: Intestinal epithelial cells (IEC) were freshly isolated from ileal tissues of 10 and 50 WK SAMP1/Yit and control mice, and cultured in the presence or absence of TNF(10 ng/ml). IEC lysates and supes were collected and measured for mRNA and protein levels (after 6 and 18 hr, respectively) of the IEC-derived cytokines, IL-18, IL-1ra and chemokines KC, JE/MCP-1 and MIP-1. Full thickness ileal tissues were processed for immunohistochemical staining of IL-1ra and IL-18. Results: JE/MCP protein levels, but not KC, were increased in TNF-induced IEC from 10 WK (stim-528$\pm$72 vs. unstim-123$\pm$20 pg/ml) compared to 50 WK (stim-54$\pm$10; unstim-39$\pm$6 pg/ml) SAMP1/Yit mice. Similarly, MIP-1 was increased in TNF-induced IEC from 10 WK (stim-27$\pm$13 vs. unstim-6$\pm$2 pg/ml) compared to 50 WK (stim-2$\pm$.7; unstim-2$\pm$.6 pg/ml) SAMP1/Yit mice. In addition, IEC-derived IL-18 was elevated in 10 WK (stim-1209$\pm$52; unstim-1243$\pm$36 pg/ml) compared to 50 WK (stim-566$\pm$27; unstim-933$\pm$70 pg/ml) SAMP1/Yit and control (10WK, stim-2090$\pm$53; unstim-1371$\pm$56 and 50 WK, stim-1376$\pm$62; unstim-1371$\pm$57 pg/ml) mice. Western Blot analysis confirmed these results. Immunohistochemical staining localized both IL-18 and IL-1ra to the epithelium, with specific expression of IL-1ra to M cells, of the SAMP1/Yit. Conclusion: A growing body of evidence supports the role of IEC as active participants during gut inflammation. Normal immunoregulatory responses of healthy IEC may be altered during disease processes which may change from protective to pathogenic. Our results indicate that IEC from SAMP1/Yit mice, compared to controls, display differential responses to potential initiating factors (*i.e.* luminal antigens), suggesting that dysregulated IEC responses may play a major role in the pathogenesis of CD.

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Intestinal Epithelial Cell (IEC) Expression of the Major Histocompatibility Complex (MHC) Class I-like Molecule, CD1d, Is Upregulated by Lumenal Components of the Gastrointestinal Tract.

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Background: CD1d belongs to a family of proteins that share structural and functional homology with the MHC class I and class II molecules. Recent studies have revealed the ability of CD1d to bind glycolipid antigens and present them to restricted subsets of T cells. CD1d expression has been detected on the surface of both professional and non-professional antigen presenting cells including IECs. However the mechanisms which regulate CD1d expression on any cell type remain to be elucidated. Aims: To investigate the possibility that expression of CD1d on IECs is influenced by factors present within the intestinal lumen, a site that is rich in bacterial-derived glycolipid antigens. Methods & Results: Soluble components of human fecal samples isolated from a range of healthy individuals were extracted in phosphate buffered saline and