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# Intestinal Flora Is Involved in the Physiologic Regulation of Colonic Paracellular Permeability: Role of Proteinase-Activated Receptor (PAR)-2

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Supplement to

# Gastroenterology



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**Digestive Disease Week  
and the  
105th Annual Meeting of the American  
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*May 15-20, 2004, New Orleans, LA***

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- Program of the Annual Meeting of the American Gastroenterological Association, the American Association for the Study of Liver Diseases, the Gastroenterology Research Group, the Society for Surgery of the Alimentary Tract, and the American Society for Gastrointestinal Endoscopy
- Abstracts of Papers Accepted by the American Gastroenterological Association
- Abstracts of Papers Accepted by the American Association for the Study of Liver Diseases
- Abstracts of Papers Accepted by the Society for Surgery of the Alimentary Tract

cludin instability & monolayer barrier disruption; 3) The ability to alter oxidation, phosphorylation, and stability of occludin cytoskeleton is a novel molecular mechanism not previously attributed to these PKC isoforms in cells

T1823

**Expression of Claudins in Inflammatory Bowel Disease**

Syam Prasad, Katri Kaukinen, Roberto Mingrino, Thomas MacDonald, Jane Collins

**Background:** In inflammatory bowel disease (IBD) intestinal permeability is increased, which is believed to result in inappropriate exposure of the gut immune system to bacterial flora. Tight junctions regulate epithelial paracellular permeability by mechanisms involving the claudin family of adhesive transmembrane proteins. We hypothesised that alterations in claudin expression occur in IBD and that inflammatory cytokines induce these changes.

**Methods:** We examined the expression of claudins 2-4 in inflamed and non-inflamed colons by immunostaining and Western blotting. The responses of gut epithelial cells to cytokines was studied using T84 cells in monolayer culture, monitoring the transepithelial electrical resistance (TER), the permeability to fluorescently-labelled 4kDa dextran and the expression of claudins 2-4 by immunofluorescence and Western blotting.

**Results:** In normal colons claudin 2 could not be detected, whereas in all cases of active IBD and most cases of inactive ulcerative colitis we observed claudin 2 expression in the tight junctions of crypt epithelial cells. By contrast, the expression of claudins 3 and 4 in normal colons was observed in both the tight junctions and the lateral cell membranes of epithelial cells, but was reduced in the surface epithelium of some cases of active disease. Western blotting confirmed these findings. T84 cells stained simultaneously with antibodies to both claudin 2 and Ki67, a marker of cells in cycle, did not show colocalisation of these two antigens. 72 hours exposure of mature T84 monolayers (TER>700ohm.sqcm) to IFN $\gamma$  (100ng/ml) and TNF $\alpha$  (1ng/ml) led to a mean 54% fall in TER relative to control monolayers and a 4-fold increase in dextran permeability, associated with falls in the expression of claudins 2 and 3 and the internalisation of claudin 4.

**Conclusions:** Claudin 2 has been proposed to contribute sodium-permeable pores to the tight junction strands of epithelia, and we have shown that its expression is increased in the crypt epithelium in IBD. This does not appear to correlate with cell division. Alterations in claudin expression may instead be a response to the increased levels of certain cytokines found in the mucosa in IBD and this may underlie the increased permeability and leak-flux diarrhoea noted in these diseases.

T1824

**Intestinal Flora Is Involved in the Physiologic Regulation of Colonic Paracellular Permeability: Role of Proteinase-Activated Receptor (PAR)-2**

Nicolas Cenac, Rafael Garcia-Villar, Christel Salvador-Cartier, Jean Fioramonti, Lionel Bueno

**Background:** The role of PAR-2 in the colon remains unclear. Our previous data showed that both in vivo and in vitro activation of colonic PAR-2 increased paracellular permeability. On another hand, colonic resident bacteria have been shown to interact with colonocytes and intercellular tight junctions by a mechanism yet to be defined. The present study was aimed at identifying relationships between PAR-2, paracellular permeability and resident flora in mouse colon. **Methods:** Ten groups of 8 Swiss mice (26-31g) were used. Groups 1 to 6 received simultaneously for 2 weeks two antibiotics in drinking water (ampicillin 0.5 g/l and neomycin 1 g/l), while groups 7-10 only received tap water. Mice were treated by intracolonic infusion with either trypsin (400 U/mouse; 50  $\mu$ l) once (2h; groups 1, 7), twice (12h and 24h; group 2), thrice (24h, 12h, and 2h; group 3), or standardized colonic content once (2h; groups 4, 8), twice (12h, 2h; groups 5, 9), before the end of antibiotic treatment and permeability measurement. Groups 6 and 10 were controls. Colonic paracellular permeability was measured by infusing <sup>51</sup>Cr-EDTA into the colon (0.7  $\mu$ Ci; 0.25 ml/h; 2 hrs), and expressed as % of radioactivity recovered outside the colon. Colons were collected in all mice for PAR-2 immunohistochemistry. Values were expressed as the mean  $\pm$  SEM for each group. **Results:** In groups untreated by antibiotics, trypsin increased paracellular permeability (8.58  $\pm$  1.13 vs. 2.08  $\pm$  0.40 %; p<0.01) and one or two infusions of colonic contents had no effect (1.64  $\pm$  0.27 and 2.32  $\pm$  0.31 vs. 1.82  $\pm$  0.33 %, respectively). In contrast, treatment with antibiotics decreased paracellular permeability (0.81  $\pm$  0.20 vs. 2.12  $\pm$  0.13 %; p<0.01) and colonocyte PAR-2 expression, as compared with controls. In antibiotic-treated mice a single infusion of trypsin or colonic contents had no effect, whereas three infusions of trypsin or two infusions of colonic contents restored paracellular permeability (2.27  $\pm$  0.36 and 1.96  $\pm$  0.17 vs. 2.12  $\pm$  0.13 %, respectively), while PAR-2 expression was similar to that of controls. **Conclusion:** Intestinal flora maintains a basal paracellular permeability in relation with PAR-2 expression. The secretion of proteases by intestinal flora, which in turn may activate PAR-2, could be involved in the physiological regulation of tight junction function.

T1825

**Epidermal Growth Factor Attenuates Acetaldehyde-Induced Reorganization of Actin Cytoskeleton and Disruption of Tight Junctions**

Parimal Sheth, Ankur Seth, S. Basuroy, R. K. Rao

Increased intestinal permeability and endotoxemia play crucial role in the alcoholic liver disease. A significant body of evidence indicates that acetaldehyde, the metabolic product of ethanol, is accumulated in the colonic lumen after alcohol consumption. Our previous study showed that acetaldehyde disrupts tight junctions (TJ), and increases paracellular permeability in Caco-2 cell monolayer. In the present study we determined the roles of cytoskeletal reorganization and phosphorylation of TJ- and adherens junction (AJ)-proteins, and evaluated the protective effect of epidermal growth factor (EGF) on acetaldehyde-induced disruption of TJ. **Methods:** Caco-2 cells on Transwell inserts were exposed to acetaldehyde (100-600 mM) in the absence or presence of EGF (3-30 nM) for 3-6 hr. Permeability was evaluated by measuring transepithelial electrical resistance (TER), and

unidirectional flux of FITC-inulin or FITC-lipopolysaccharide (LPS). Reorganization of actin cytoskeleton and redistribution of TJ and AJ proteins were determined by confocal microscopy. Actin binding of TJ- and AJ-proteins analyzed by immunoblot analysis in isolated F-actin fraction. Phosphorylation of TJ- and AJ-proteins on Tyr, Ser and Thr residues was analyzed by immunoprecipitation and immunoblot analysis. **Results:** EGF reduced acetaldehyde-induced reduction of TER and increase in permeability to inulin and LPS, in a time- and dose-dependent manner. EGF-mediated protection was effective both at the apical and basolateral surface, and was attenuated by AG1478, specific inhibitor of EGF-receptor kinase. Acetaldehyde induced reorganization of actin cytoskeleton, and resulted in redistribution of occludin, ZO-1, E-cadherin and  $\beta$ -catenin from the cellular junctions, and reduced the levels of occludin, ZO-1, E-cadherin and  $\beta$ -catenin associated with the F-actin. EGF reduced these effects of acetaldehyde on actin cytoskeleton and TJ/AJ proteins, and their interactions. Acetaldehyde increased phosphorylation of occludin, ZO-1, E-cadherin and  $\beta$ -catenin on Tyr residues, and decreased phosphorylation of Ser/Thr residues; and EGF prevented these effects of acetaldehyde. **Conclusion:** These results indicate that EGF attenuates acetaldehyde-induced disruption of TJ and AJ by reducing acetaldehyde-induced effects on the actin cytoskeleton, phosphorylation of TJ- and AJ-proteins, and release of TJ- and AJ-proteins from the actin cytoskeleton. Support by DK55532 and AA12307.

T1826

**Mast Cells Induce Epithelial Barrier Dysfunction by Activation of Protease-Activated Receptor 2 (PAR2)**

Eileen F. Grady, Ping-Chang Yang, Silvia Amadesi, Claire Jacob, Graeme S. Cottrell, Pam Singh, Dalila Darmoul, Mary Perdue, Nigel W. Bunnett

Mast cells are increased in the intestinal mucosa during allergy, stress, irritable bowel syndrome and inflammatory bowel disease. Mast cells mediate increased mucosal permeability during stress and inflammation by unknown mechanisms. **Aim:** We examined if tryptase, the major protease of human mast cells, regulates epithelial permeability by cleaving and activating PAR2. **Methods:** We determined: 1) the expression and activation of PAR2 in colonocytes (NCM460 and T84) by RT-PCR and by measuring [Ca<sup>2+</sup>]<sub>i</sub> using fura2; 2) the permeability of epithelial monolayers indicated by transepithelial resistance (TER) and flux of horseradish peroxidase (HRP) after adding HMC-1 mast cells (activated by 48/80), lysates or proteases; 3) pathways and mechanisms using receptor desensitization, specific inhibitors and imaging. **Results:** Both colonocyte cell lines expressed mRNA for PAR2. PAR2 was functional, as indicated by increased [Ca<sup>2+</sup>]<sub>i</sub> in response to tryptase, trypsin and coagulation factor Xa. Pretreatment with PAR2-activating peptide (PAR2-AP, corresponds to tethered ligand domain) desensitized responses, suggesting that proteases signal by cleaving PAR2. Mast cell lysate also increased [Ca<sup>2+</sup>]<sub>i</sub> in epithelial cells, and responses were reduced by tryptase inhibitor. Mast cells, proteases or PAR2-AP added to the basolateral compartment of Transwells decreased TER and induced uptake of HRP into enlarged endosomes. These effects were also diminished by tryptase inhibitor. PAR2 activation induced redistribution of proteins from tight junctions, including ZO1, occludin and claudin, and caused a reorganization of cortical actin. PAR2 activation stimulated activity of extracellular signal regulated kinases (ERK) 1 and 2 in T84 cells which was prevented by down-regulation of b-arrestins using RNAi. This suggests that b-arrestins recruit PAR2 and ERK1/2 to endosomes and thereby specify the localization and function of activated ERKs. Finally, an inhibitor of ERK1/2 formation prevented PAR2-stimulated increases in epithelial permeability. **Conclusions:** Mast cell proteases signal to colonic epithelial cells by activating PAR2. This activation promotes reorganization of proteins from tight junctions and increases epithelial permeability that is mediated by b-arrestins and ERK1/2. These findings have implications for intestinal conditions where enhanced permeability contributes to the disease process. Supported by DK43207 and C1HR.

T1827

**Involvement of the Proteinase-Activated Receptor 2 (PAR-2) in the Zot/zonulin-Mediated Regulation of Intestinal Tight Junctions**

Maria Grazia Clemente, Stefanie N. Vogel, Morley Hollenberg, Stefano De Virgili, Alessio Fasano

**Background:** We have recently showed that zonulin, the eukaryotic Zonula occludens toxin (Zot) analogue that modulates intercellular tight junctions (tj), is structurally similar to mast cell protease (MCP)-II. Further, the active Zot fragment  $\Delta$ G N-terminus contains a motif structurally similar to the tethered ligand agonist of proteinase-activated receptor (PAR)-2, SLIGKV-amide. **Aim:** To establish whether PAR-2 (or a related receptor) represents the target receptor for both Zot and zonulin. **Methods:** Human intestinal epithelial cells (Caco2) were incubated with either FITC-labelled Zot/zonulin binding antagonist FZ1/0 peptide or with mouse monoclonal anti-human PAR-2 antibodies, followed by incubation with rhodamine-labelled anti-mouse IgG antibodies. Similar experiments were conducted in situ on duodenal biopsies obtained from patients with normal intestinal biopsies. The effect of SLIGKV-amide on the intestinal cell cytoskeleton was assessed both in the presence and absence of FZ1/0 in Caco2 cells stained with FITC-phalloidin. The effect of FZ1/0 on SLIGKV-amide-induced changes in transepithelial electrical resistance (TEER) was also tested on rat intestinal tissues mounted in the microsnapwell system. **Results:** Immunofluorescent particles were visualized in Caco2 cells with both FITC-FZ1/0 and anti-PAR-2 antibodies. Overlapping of the two images showed colocalization of PAR-2 and FZ1/0. Similar results were obtained with in situ immunofluorescence experiments performed on human intestinal biopsies. In binding-competition experiments, cells exposed to an excess of SLIGKV-amide ligand showed a significant reduction of FZ1/0 immunofluorescent staining particles compared to monolayers exposed to a scrambled peptide, suggesting that FZ1/0 binds to a site very close to or synonymous with PAR2. Activation of PAR2 in Caco2 cells with SLIGKV-amide induced a significant intracellular cytoskeleton reorganization in Caco2 cells that was prevented by pre-treatment with FZ1/0 peptide. Addition of SLIGKV-amide to the luminal aspect of the intestine decreased TEER compared to untreated tissues and these changes were completely prevented by pretreatment with FZ1/0. **Conclusions:** Our findings suggest that PAR-2 (or a closely related moiety) could be the target receptor for both Zot and zonulin and is involved in the regulation of intercellular tj.