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## Colonic luminal proteases regulate colonocyte PAR-2 expression and paracellular permeability in mice

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Supplement to

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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

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iNOS, by western blot), CCPR (Bromodeoxyuridine counts/crypt), and crypt apoptotic activity (Caspase-3 + cells, both BRDU and Caspase-3 by immunohistochemistry) RESULTS: GLP-2 with established inflammation improved animal weights, reduced MPO and cytokine production (Table 1), but CCPR was not increased. CONCLUSIONS: GLP-2 is anti-inflammatory in TNBS ileitis, but this effect does not occur from increased CCPR. Further studies are indicated to determine the mechanisms of these effects, but suggest a relationship between inflammation suppression, and the regulation of nutrient absorption.

Groups	Δ body Weight (g)	MPO (units)	IFN γ (OD/mm <sup>2</sup> )	IL-1β (OD/mm <sup>2</sup> )	iNOS (OD/mm <sup>2</sup> )	CCPR BrdU + cells/crypt	Apoptosis (caspase-3 + cells/crypt)
Controls	1.0 ± 2.8	2.14 ± 0.28	100 ± 6	100 ± 6	100 ± 15	13.8 ± 1.7	8.3 ± 0.6
TNBS	-25 ± 2.7	12.2 ± 1.6	135 ± 5	240 ± 35	185 ± 17	30.3 ± 1.5	25.6 ± 1.3
D-GLP-2	-7.8 ± 0.8*	5.2 ± 0.6*	112 ± 4*	150 ± 20*	110 ± 15*	17.0 ± 1.3*	5.9 ± 0.4*

(Data: Mean ± SEM, \* p < 0.05 vs TNBS by ANOVA)

T1531

Colonic Luminal Proteases Regulate Colonocyte PAR-2 Expression and Paracellular Permeability in Mice

Nicolas Cenac, Richard Roka, Julien Demaude, Christelle Salvador-Cartier, Rafael Garcia-Villar, Jean Fioramonti, Lionel Bueno

**Background:** The physiological role of proteinase-activated receptor-2 (PAR-2) located on colonocytes remains unclear. Their stimulation by intracolonic infusion of PAR-2-activating peptide (SLIGRL) increases colonic paracellular permeability (CPP) by a direct MLCK-dependent mechanism. Resident bacteria may affect the level of luminal protease activity modulating the degree of activation of PAR-2 and subsequently the CPP. The present study aimed to evaluate the role of colonic microflora in PAR-2 responsiveness of colonocytes both in vivo and in vitro and its influence on colonocyte PAR-2 expression.

**Methods:** Six groups of Swiss mice and 2 groups of C57BL6 mice were used for in vivo and in vitro studies respectively. Three groups of Swiss mice and 1 group of C57BL6 mice were treated for 12 days with antibiotics (AB) (0.5 g/L ampicillin and 1 g/L neomycin in drinking water). In the in vivo study, 2 groups (1 control and 1 AB) were sacrificed at day 12 for measurement of serine-protease activity in colonic luminal contents and immunohistochemistry of PAR-2 in colonic mucosa. Four groups (2 controls and 2 AB) were treated daily with intracolonic administration (50 μL) of trypsin (400 U) or saline. At day 12, these groups were injected intracolonic with SLIGRL (5 μg) or its vehicle. Permeability was assessed in these 4 groups during 4 hours after SLIGRL or vehicle treatment by determining the percentage of <sup>51</sup>Cr-EDTA crossing the colonic barrier. In the in vitro study, permeability of colonic strips and the effect of SLIGRL (25 μM) were investigated by determination of dextran-flux in Ussing chambers both in control and in AB C57BL6 mice.

**Results:** In mice treated with AB the serine-protease activity was reduced as compared with controls (9.60 ± 9.03 vs. 48.47 ± 9.50 U of trypsin) coupled with a decrease in PAR-2 expression but with no change in CPP (0.84 ± 0.04 vs. 1.05 ± 0.17 %). In AB-treated mice, intracolonic SLIGRL did not increase CPP and daily trypsin administration restored both PAR-2 expression and SLIGRL responsiveness. In vitro, the effect of SLIGRL on permeability was also reduced by 80.5% in AB treated mice (0.52 ± 0.54 vs. 2.66 ± 0.72 nmol/h/cm<sup>2</sup>).

**Conclusion:** Our data evidence that colonic microflora affects colonocyte tight junction response to PAR-2 activation by affecting its expression. Therefore, the serine-protease activity of colonic content may play an important role in the control of gut immune mucosal barrier.

T1532

IL-8 Production From Human Intestinal Epithelial Cells Mediated By Protease-Activated Receptor

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**Background and Aims:** It has been reported that some proteases like thrombin, trypsin and trypsinase increase in intestinal lumen and intestinal mucosa of patients with inflammatory bowel disease. Recently, some serine proteases have been proposed to play important roles in the inflammatory and immunological reaction through four specific protease-activated receptors (PAR)1-4. In the present study, we investigated the PAR expression on intestinal epithelial cells and IL-8 production via PAR-mediated mechanisms. **Methods:** Human intestinal epithelial cell line HT29 cells were treated with thrombin (1-100 U/ml) as natural PAR1 agonist, trypsin (0.1-10nM), trypsinase (0.01-1.0 U/ml) as natural PAR2 agonists, a synthetic PAR1 agonist (SFLLR amide, 0.05-1.0mM) or a synthetic PAR2 agonist (SLIGKV amide, 0.01-0.5mM). IL-8 mRNA and IL-8 protein were then measured by reverse-transcriptase polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA), respectively. We also examined the expression of PAR1 and PAR2 on HT29 cells by RT-PCR, immunostaining, and western blotting after treatment of HT29 cells with or without IL-1 beta(20U/ml). In addition, we assessed the inhibitory effects of anti-PAR1 and PAR2 blocking antibody on IL-8 production from HT29 cells stimulated by thrombin and trypsin, respectively. **Results:** The treatment of HT29 cells with thrombin, trypsin, trypsinase, synthetic agonists SFLLR amide or SLIGKV amide resulted in the production of IL-8 protein and IL-8 mRNA in a dose- and time-dependent manner. Anti-PAR1 and PAR2 blocking antibody inhibited the increased production of IL-8 by thrombin and trypsin, respectively. PAR1 and PAR2 mRNA were constitutively expressed on HT29 cells. The presence of PAR1 and PAR2 protein on HT29 cells was confirmed by immunostaining or western blotting. In addition, the expression of their proteins and mRNAs were increased by stimulation of IL-1beta. **Conclusions:** These data indicate that thrombin, trypsin or trypsinase induces the production of IL-8 through PAR1 and PAR2 on human intestinal epithelial cells, suggesting central role of protease/PAR-mediated inflammatory reaction in colonic damage.

T1533

Effect of Protease Activated Receptor-2 Activation on Dog Pancreatic Duct Epithelial Cell Transepithelial Resistance

Toan D. Nguyen, Thomas Wong

**Background:** Protease-activated receptors (PAR) are G-protein-coupled receptors that are activated following proteolysis rather than through binding to an extracellular ligand. Of the four PARs identified, PAR-2 and PAR-4 are activated by pancreatic trypsin. We previously demonstrated that PAR-2, located on the laterobasal surface of dog pancreatic duct epithelial cells (PDEC), induced a [Ca<sup>2+</sup>]<sub>i</sub> increase to stimulate Ca<sup>2+</sup>-activated K<sup>+</sup> and Cl<sup>-</sup> channels (Nguyen et al., JCI 103: 261-269, 1999). Because of recent observations that PAR-2 alters colonic paracellular permeability, we examined the effect of PAR-2 activation on the transepithelial resistance (TER) of PDEC. **Methods:** Polarized PDEC, obtained from the main pancreatic duct of a dog, were cultured on Transwell inserts over a feeder layer of human gallbladder myofibroblasts. The PAR-2 activating peptide (AP, amino acid sequence: SLIGRL), corresponding to the tethered ligand of mouse PAR-2, was used to activate these receptors on PDEC. Transepithelial resistance was measured, using an epithelial voltohmmeter (EVOM) before and throughout treatment with 10 μM of either AP or RP, the peptide with the reversed amino acid sequence of AP, LRGILS. **Results:** Treatment with 10 μM serosal AP induced a transient decrease in TER to 57 ± 2 % of the starting value (995 ± 11 Ω.cm<sup>2</sup>, n = 3) after 2 min. This TER however recovered quickly to 83 ± 6 % of the starting value after 15 min, and to 107 ± 5 % after 24 hours. In contrast, no decrease in TER was noted when AP was added to the luminal compartment, or when RP was added to either the serosal or luminal compartment. Of note, this transient TER decrease parallels the previously described transient lsc increase stimulated by serosal PAR-2 agonists on these PDEC. **Conclusion:** The effect of serosal AP on TER is most likely mediated by the PAR-2 previously localized to the laterobasal membrane of these PDEC, since it was not reproduced by luminal AP or RP. Because of the parallel decrease in TER and increase in lsc, further investigations are needed to determine the relative contributions of the transcellular and paracellular permeabilities to this transient TER decrease. On the other hand, no long lasting effect of continuous and direct PAR-2 activation on the TER of PDEC was observed. Funded by NIH RO1 DK55885 and VA Merit Review.

T1534

PAR-2-Induced Epithelial Chloride Secretion Involves the Epidermal Growth Factor Receptor and a Map Kinase Signaling Pathway

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Proteinase-activated receptor (PAR)-2 is activated by trypsin-like serine proteinases and selective synthetic peptides. It has been implicated in intestinal inflammation, but its role in the regulation of intestinal mucosal function remains unclear. We studied the stimulus-secretion coupling mechanisms of PAR-2 activation on epithelial anion transport in a cell culture model. **Methods:** 1. RT-PCR and immunocytochemistry were used to determine PAR-2 expression in SCBN intestinal epithelial cells. 2. SCBN monolayers were grown on Snapwell supports and mounted in modified Ussing chambers. Short circuit current (Isc) was monitored as a measure of net electrogenic ion transport. Concentration response curves to the PAR-2 activating peptides SLIGRL-NH<sub>2</sub> and 2-furoyl-LIGRLO-NH<sub>2</sub> were determined. Polarity of the receptor was determined by either apical or basolateral addition of SLIGRL-NH<sub>2</sub>. The role of chloride ion, calcium, prostaglandins, MAP kinases and the EGF receptor in the response to PAR-2 activation by SLIGRL-NH<sub>2</sub> was determined using specific inhibitors (indomethacin, verapamil, thapsigargin, PD98059, PD153035) and/or buffers (chloride free). **Results:** 1. RT-PCR and immunocytochemistry showed PAR-2 expression in SCBN cells. 2. Basolateral, but not apical, exposure to either SLIGRL-NH<sub>2</sub> or 2-furoyl-LIGRLO-amide resulted in a concentration-dependent increase in Isc indicating a polarized distribution of PAR-2 in SCBN cells. Experiments conducted in chloride free buffer resulted in an 88% decrease in the response to PAR-2 activation (p < 0.001). Thapsigargin, but not verapamil, pretreatment resulted in a significant decrease in PAR-2-AP-induced chloride secretion (89%, p < 0.001) indicating that increasing calcium from intracellular stores and not external sources was involved in the response. PD98059 pretreatment significantly reduced responses to PAR-2 activation (63%, p < 0.001) as did pretreatment with PD153035 (82%, p < 0.001), implicating EGF receptor tyrosine kinase and MAP kinase pathways. Indomethacin pretreatment also resulted in a significantly lower Isc in response to PAR-2 activation (65%, p < 0.05) suggesting that the response was also dependent upon cyclooxygenase activity. **Conclusions:** PAR-2 activation induces epithelial chloride secretion that is regulated by the release of calcium from intracellular stores. The stimulus-secretion coupling pathway appears to be mediated by an EGF receptor-ERK1/2 signaling pathway and cyclooxygenase activity.

T1535

Overexpression of Beta-Catenin Enhances Proteinase-Activated Receptor-1-Induced Cell Proliferation in Colon Cells

Hongying Wang, Morley D. Hollenberg, Wallace K. MacNaughton

Proteinase-activated receptors (PARs) are G protein-coupled receptors that are activated by serine proteinases which cleave the extracellular N-terminus to reveal a new N-terminus which acts as a "tethered ligand" to activate the receptor. PAR-1, also known as the thrombin receptor, plays an important role in wound healing and inflammation. Increasing evidence has shown that PAR-1 is also involved in tumor progression. β-catenin has been well described as a crucial tumorigenic determinant in many colon cancers. However, nothing to date has been reported on the interaction between PAR-1 and β-catenin. We hypothesize that overexpression of β-catenin may affect the proliferative response of colonic cells to the activation of PAR-1. **Methods and Results:** RKO, a colonic cancer cell line with low levels of β-catenin, was used. We transfected constitutively activated β-catenin into RKO cells and made an RKO-β-catenin stably transfected cell line (RKO<sub>cat</sub>). When we stimulated cells with the PAR-1-activating peptide, TFLLR-NH<sub>2</sub>, RKO<sub>cat</sub> showed increased sensitivity with respect to the characteristic PAR-1-induced calcium influx signal compared to the RKO parental cell line. The maximum effect in RKO<sub>cat</sub> cells was more than 2-fold higher (p < 0.05) than