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Supplement to

# Gastroenterology



*Garcia*

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

- 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

NOD2 as detected by real-time quantitative RT-PCR, gene reporter assays and western blotting. In contrast, IFN- $\gamma$  suppressed TNF- $\alpha$ -induced expression of the intestinal cytoprotective enzymes COX-1 and COX-2 while having no effect on the up-regulation of the anti-apoptotic protein cIAP2. Intriguingly, the addition of PGE<sub>2</sub> - the major prostaglandin metabolite resulting from COX-1 and COX-2 activity - protected against cytokine (IFN- $\gamma$  + TNF- $\alpha$ ) mediated apoptosis. Conclusions: Collectively, these data indicate that IFN- $\gamma$  differentially modulates TNF- $\alpha$ -induced NF- $\kappa$ B target gene expression in intestinal epithelial cells. The observation that IFN- $\gamma$  suppressed induction of the intestinal cytoprotective enzymes COX-1 and COX-2 indicates that they are important anti-apoptotic NF- $\kappa$ B target genes in this cell type. Further studies will investigate the involvement of the STAT1 and IRF-1 transcription factors in the modulation of NF- $\kappa$ B target gene regulation by IFN- $\gamma$ .

S1031

# **Epithelial TLR5 Is Constitutively Focalized to the Mouse Cecum And Exhibit a Distinctive Down-Regulation During Experimental Colitis** Cesar F. Ortega-Cava, Shunji Ishihara, Yoshikazu Kinoshita

We have previously shown that innate pattern recognition receptors TLR2, TLR4 and CD14 are strongly expressed in the mouse colon epithelium in a compartmentalized manner. Mapping of toll like receptors (TLR) in the gut may be important since colon inflammatory diseases have regional particularities. Now we aim to localize TLR5, the receptor for bacterial flagellin. Normal and dextran sodium sulfate (DSS)-colitic BALB/c mice guts were explored. TLR5 mRNA was preferentially expressed at the cecum epithelium of normal mice as shown by northern blotting of RNA extracted from epithelial scrapings and detached crypts. Laser-capture microdissection coupled to RT-PCR further confirmed TLR5 mRNA localization. TLR5 protein was in agreement with mRNA as evidenced by western blotting. The modulation of TLR5 during 2.5% DSS-induced colitis was studied in vivo. Colitis was located at the distal colonic segment causing and increase of IL-1 beta expression. Interestingly, while TLR2, TLR4 and CD14 were up-regulated in the inflamed colon, TLR5 was down-regulated at both mRNA and protein levels at the cecum. The decreased expression was more evident as DSS-colitis was cycled causing chronic colitis. Thus, regulation of TLR5 by proinflammatory cytokines may differ from other TLRs. In addition, during the recovery phase of acute DSS colitis, TLR5 expression was transiently increased at day 4, when regeneration occurs. Studies on Colon 26 mouse cell line showed that TNF alpha and IFN gamma down-regulate and TGF alpha up-regulate TLR5. In conclusion, TLR5 is constitutively restricted to the mouse cecum epithelium and efficiently down-regulated during DSS colitis indicating that may not be involved in the pathogenesis of this colitis model. TLR5 expression regulation differs from other TLRs. The mouse cecum appears to have particular functions in relation to flagellated bacteria.

S1032

# **Peroxisome Proliferator-Activated Receptor-Gamma (PPAR $\gamma$ ) a Pivotal Key for Pro-Inflammatory Effect of Leptin on Human Epithelial Colonic Cells** Mohammad Albohassani, Jean-Claude Marie, Hassan Ashktorab, Andre Bado, Iradj Sobhani

**Background & Aim.** Leptin is a multifunctional hormone that regulates body weight homeostasis, neuroendocrine and immune functions. It plays an active role in innate and adoptive immunity through alteration of various target gene transcription. Here we analyze direct effects of leptin on cytokine production from human epithelial colon cells. **Materials & Methods:** Fresh epithelial cells isolated from normal colonic mucosa (N=3), HT-29 and Caco-2 human colonic epithelial cell lines in confluent culture were used. The effects of leptin alone or in association with PPAR $\gamma$  agonist (Rosiglitazone & MCC-555) or antagonist (GW-9662), on the production of pro- (IL-8, MCP1, IL-6, TNF $\alpha$ ) and anti- (IL-10) inflammatory cytokines were determined by means ELISA and Ribonuclease Protection Assay (RPA). Activity of NF- $\kappa$ B was measured using luciferase reporter gene assay upon leptin challenge. To inhibit NF- $\kappa$ B activity in situ, cells were transfected by a dominant negative I $\kappa$ B super-repressor and NF- $\kappa$ B activity, cytokines and PPAR $\gamma$  proteins were measurements. The amounts of PPAR $\gamma$  proteins was analyzed by Western blot. **Results:** Leptin increased the release of IL-8 and MCP1 but unaffected both basal and LPS-stimulated IL-6 and TNF $\alpha$  in the supernatant of confluent normal human epithelial fresh colonic, HT29 or Caco-2 cells. Up to 24 hours after cells exposure to leptin, IL-8 significantly increased and IL-10 decreased, and these effects were dose- and time-dependently documented in HT29 and Caco-2 cells. At the same time, leptin increased the luciferase activity of NF- $\kappa$ B in a dose-dependent manner. In cells transfected by the dominant negative I $\kappa$ B super-repressor, IL-8 and MCP1 productions (ELISA & RPA) and NF- $\kappa$ B activity failed to increase in response to leptin. In addition, leptin-treated cells displayed a reduction amount in PPAR- $\gamma$  protein. Furthermore, treatment of the cells with Rosiglitazone and MCC-555, induced a significant decrease in IL8 and MCP1 production but unaltered the activity of NF- $\kappa$ B. The PPAR $\gamma$  antagonist (GW-9662) has no inhibitory effect on cytokine production while NF- $\kappa$ B activity was altered by neither PPAR $\gamma$  agonists nor antagonists. **Conclusion:** We suggest that leptin induces a pro-inflammatory cytokine response in colon epithelial cells. Although NF- $\kappa$ B and PPAR $\gamma$  are involved in this effect, we show that PPAR $\gamma$  suppression rather than NF- $\kappa$ B activity is required for cytokine productions.

S1033

# **Adenosine Is a Negative Regulator of NF-kappaB and MAPK Signaling in Intestinal Epithelial Cells**

Humberto B. Jijon, Judith Altarejos, John Walker, Karen L. Madsen

**Rationale:** The levels of adenosine in extracellular fluids during inflammation can reach millimolar concentrations as a result of secretion and cellular leakage during necrosis. Previous studies suggest adenosine possesses anti-inflammatory properties however, the mechanisms by which adenosine affects immune function remain unclear, particularly in

the intestine. In the present study we hypothesized that adenosine directly affects proinflammatory gene expression in intestinal epithelial cells (IECs) through modulation of NF- $\kappa$ B signaling.

**Methods:** HT-29 cells were treated for 30 mins with 100-1000  $\mu$ M adenosine prior to incubation with various stimuli. Monolayers and supernatants were harvested and IL-8 mRNA expression and secretion measured by RT-PCR and ELISA respectively. NF- $\kappa$ B activation was measured by EMSA, Western blotting and reporter assays. MAPK activation was monitored via Western blotting.

**Results:** Adenosine (250-1000 uM) pretreatment resulted in a profound reduction in IL-8 expression and secretion (20-80%) in response to TNF (10 ng/ml). This was paralleled by inhibition of  $\kappa$ B-driven luciferase expression and NF- $\kappa$ B DNA binding but not by changes in I $\kappa$ B degradation or p65 phosphorylation. Similarly, adenosine inhibited IL-8 secretion in response to 5 ng/ml IL-1, 200 nM LPS and 100 nM PMA, but not 10 mM hydrogen peroxide. Adenosine receptor agonists/antagonists failed to promote or prevent NF- $\kappa$ B inhibition respectively. On the other hand, nucleoside transporter blockade partially restored TNF-induced NF- $\kappa$ B activation, suggesting that the effects of adenosine are mediated intracellularly. Adenosine kinase inhibition failed to reverse the effects of adenosine suggesting that intracellular conversion of adenosine to AMP is not required. Likewise, inosine (0.1-5 mM) did not mimic the effects of adenosine suggesting that adenosine catabolism is not involved. Interestingly, adenosine pretreatment also resulted in reduced ERK, p38 and JNK MAPK phosphorylation following TNF treatment.

**Conclusions:** Adenosine targets both NF- $\kappa$ B signaling and the activation of MAPKs leading to a reduction in NF- $\kappa$ B activity and gene expression. These effects occur independent of surface adenosine receptors and may involve uptake through nucleoside transporters. This study identifies adenosine as a potent negative regulator of proinflammatory signaling in IECs and suggests the existence of a novel intracellular adenosine signaling pathway.

S1034

# **Changes in Colonic Paracellular Permeability Are Associated with Airway Challenge in Ovalbumin Sensitized Mice**

Helene Eutamene, Nicolas Cenac, Valerie Tondereau, Vassilia Theodorou, Rafael Garcia-Villar, Jean Fioramonti, Lionel Bueno

Studies have shown that a large proportion of inhaled antigen is rapidly translocated into the gastrointestinal tract. Moreover, clinical studies reveal that asthma is often associated in infants with diarrhea and/or abdominal pain. An increase in small intestinal paracellular permeability has been described in patients with type 2- and IgE-associated pathologies such as atopy and asthma. The aim of this study was to evaluate colonic permeability changes linked to an airway ovalbumin challenge in ovalbumin sensitized mice. **Methods:** Groups of 6-week old BALB/c male mice were used. Mice received on day 1 and 7 a IP administration of NaCl 0.9% or a solution containing 5  $\mu$ g/mL of ovalbumin mixed with 20 mg/mL of AL(OH)3. One week later mice were challenged by nebulisation of sterile NaCl 0.9% or 5% ovalbumin solution. 1h, 4h, 24h, 48h and 72h post challenge, colonic paracellular permeability was measured by infusing <sup>51</sup>Cr-EDTA into the colon (0.7 mL; 0.25 mL/h; 2 h), and expressed as percent of radioactivity recovered outside the colon. In parallel, 48 and 72h post challenge, the number of leucocytes and individual white cells were determined in broncho-alveolar lavages (BALs). **Results:** In sensitized mice in absence of challenge, the basal permeability was similar to controls. For the times of 1, 4 and 24 h after challenge no modification in colonic paracellular permeability was measured; however, 48h after challenge a significant increase in colonic permeability was observed ( $2.36 \pm 0.25$  vs  $0.92 \pm 0.25$  %;  $p < 0.05$ ), and at 72 h after challenge colonic permeability was restored ( $0.63 \pm 0.1$  %;  $p > 0.05$  compared with controls). In BALs the total number of leucocytes was significantly higher 48 h and 72 h after challenge in sensitized mice compared with controls (174 and 143 % increase;  $p < 0.05$ ). Likewise, the number of eosinophils was dramatically increased in BALs from mice 48h and 72h after challenge. **Conclusion:** Our data show that an airway challenge in ovalbumin sensitized mice induce a postponed increase in colonic paracellular permeability appearing after 48 h with concomitant dramatic eosinophil infiltrations in the lung. These results suggest that airway-inhaled antigens in sensitized animals triggers gut inflammatory response characterized by mucosal leakage favorizing colonic flora translocation.

S1035

# **Escherichia Coli (EC) Isolates from Crohn's Disease (CD) Tissues Are More Often Clonal, Motile and Associated with Single Flagellar Types Than EC from Control or Ulcerative Colitis (UC)**

Ying Mao, Chengru Zhu, Katherine Davis, Edgar C. Boedeker

**Background:** Expression of flagellae by enteropathogenic and enterohemorrhagic EC strains yields an inflammatory phenotype which induces IL-8 production via toll-like receptor 5 interactions with epithelial cells. **Design:** To determine whether the presence of flagellae, or of particular flagellar types, characterizes EC clones from intestinal tissues of patients (pts) with inflammatory bowel disease (IBD) we cultured resected intestinal specimens from pts with CD, UC or other diseases (carcinoma, adenoma, carcinoid, diverticulitis) for EC then tested individual colonies for motility in soft agar. Clonality of the isolates was established by RFLP/PCR analysis of their Enterobacterial Repeat Intergenic Consensus (ERIC) sequences. Flagellae were typed by RFLP/PCR analysis of the major structural flagellar gene FlhC gene. **Results:** 366 EC isolates from 166 tissues from 86 patients (38 CD, 37 UC, 11 other) were analyzed. EC were isolated from 11 CD pts (34%) #151 isolates (avg 13.6/pt); 12 UC pts (35%) #153 isolates (avg 11.8/pt); and 5 control pts (42%) #62 isolates (avg 12.4/pt). EC from CD pts were clonal yielding only 13 ERIC types (1.1 ET/pt, range 1-2 ET/pt, avg 1.16 isolates/ET). In contrast ( $p = .04$ , CD vs UC) EC from UC were more variable yielding 40 ETs (average 3.1 ET/pt, range 1-10 ET/pt, avg. 3.8 isolates/ET). Control (C) isolates were also variable ( $p = .002$ , CD vs C) yielding 16 ERIC types (average 3.2 ET/pt, avg 3.2 isolates/ET). 80% of CD EC were motile, in contrast to only 52% of UC EC and 44% of control EC. CD EC demonstrated only 11 flagellar types, avg 1/pt. In contrast UC EC had 20 flagellar types (avg 1.43/pt) and control EC had 11 flagellar types (avg 2.2/pt) ( $p = .045$  CD vs UC;  $p = .002$  CD vs C, UC vs C = NS). **Summary:** EC isolates from resected tissues of