proliferation and modulates intestinal epithelial cell proliferation and migration. We previ-ously reported that intravenous bolus injections of recombinant human HGF (rh-HGF) ameliorates experimental colitis. Therefore, HGF is a potential therapeutic agent for IBD. However, continuous systemic administration of HGF may accelerate tumorigenesis. To avoid these possible effects, local administration of HGF is desirable. The present study investigates the safety and efficacy of rectally administered rh-HGF for experimental colitis. Methods: 2,4,6-trinitrobenzene-sulfonic acid (TNBS) colitis and dextran sulfate sodium (DSS) colitis were induced in Wistar rats. The rats were administered rectal enemas of rh-HGF or phosphate-buff ered saline (PBS) once a day for seven days. Disease activity was assessed endoscopically and histologically. The proliferative activity of the colon epithelium was assessed by immu-nohistochemical detection of Ki67 antigen. Serum rh-HGF levels were measured by ELISA. Results: Rats with TNBS colitis and DSS colitis that were repeatedly administered enemas of rh-HGF showed significant reductions in the area of colonic mucosal damage and the histologic score compared to those treated with PBS. Repeated enemas of rh-HGF and intrarectal butyl injections had equivalent efficacy and induced comparable epithelial cell proliferation. In addition, rats administered repeated enemas had no detectable serum rh-HGF levels. Conclusions: Repeated enemas of HGF accelerates colonic mucosal repair and reduces the infiltration of inflammatory cells in rat experimental colitis without increasing serum levels of MMPs. Rectal administration of HGF may be a potential novel and safe therapy for IBD.

M1828

Metallloprotease Activity and Epidermal Growth Factor Receptor Transactivation are Required for PAR2-Induced COX-2 Expression in Caco-2 Cells

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Background: Protease activated receptors (PARs 1-4) are a family of G-protein-coupled receptors that are activated by N-terminal cleavage by serine proteases. PAR2, activation has been shown to stimulate matrix metalloprotease (MMP)-9 release as well as COX-2 expression in epithelial cells. PARs are also known to transactivate the epidermal growth factor receptor (EGFR). We hypothesize that MMP-dependent activation of EGFR contributes to the PAR2-mediated cyclooxygenase (COX-2) expression observed in a colon cancer cell line. Methods: Caco-2 cell monolayers were treated with the PAR2-activating peptide 2-3BRLG (IIL), plus or minus inhibitors of EGFR tyrosine kinase activity (PD133036, metallloprotease (marmastat), ERK 1/2 (PD98059 or U0126) or Src tyrosine kinase activity (PP2), for seven times with treatment with IIL. Results: IIL alone decreased COX-2 expression. Protein was collected from the cells, separated on denaturing gels, transferred to nitrocellulose membranes and blotted for COX-2, phospho-src, or the housekeeping protein actin. In some experiments, supernatants from treated cells were collected and incubated with a fluorescent MMP substrate peptide to assess levels of MMP activity. Results: COX-2 expression in Caco-2 cells was increased more than 3-fold (p<0.05) by treatment with IIL for 3 h. This increase was abrogated by treatment with PD133036, attenuated 58% (p<0.05) by co-treatment with 3 μM marmastat, but was not significantly altered by either ERK inhibitors or by selective Src tyrosine kinase inhibition. Treatment of cells with IIL for 30 min produced a 2.5-fold increase (p<0.05) in MMP activity in the culture supernatant compared to untreated control cells. Treatment for 40 min with IIL led to an increase in tyrosine phosphorylation of a protein matching the size of EGFR. Treatment with IIL also increased the tyrosine phosphorylation of this same band, an event that was prevented by co-treatment with the EGFR inhibitor. Conclusions: PAR2-stimulated COX-2 expression in Caco-2 cells requires EGFR transactivation, which may occur through shedding of an EGFR ligand by an as-yet unidentified metalloprotease (potentially a MMP). The ERK MAPK pathway and Src tyrosine kinase activity were not involved in PAR2-stimulated COX-2 expression. To our knowledge, this is the first study linking PAR2 activation to COX-2 expression in a colon epithelial cell line. The requirement for metalloproteases and EGFR in this process further emphasizes its potential relevance to colon cancer.

M1829

Colonic Mucosal A2BR Mediates Pro-Inflammatory Activities

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Background: The adenosine 2B receptor (A2B) is one of four adenosine receptors and is highly expressed as the predominant adenosine receptor in the intestinal epithelium. Immune cells express multiple adenosine receptor subtypes. We recently demonstrated that A2BR blockade with a polyclonal anti-concanavalin A antibodies, that has minimal systemic absorption, ameliorates colitis induced by DSS, or colitis that occurs spontaneously in IL10-/- mice. Similarly, A2BR-/- mice are protected from DSS, Salmonella typhimurium and TNBS-induced colitis. In the current study, we examined the role of adenosine and mucosal A2BR receptors in mediating colonic inflammation in WT mice. Bone marrow chimeras were created by transplanting bone marrow from donor mice to lethally irradiated recipients. Chimerism was assessed 5 weeks after transplantation. Colitis was induced using 3% DSS in drinking water. Mice were followed for body weight, diarrhea and blood in stool and sacrificed 7 days after induction of colitis. Mice were followed for body weight, diarrhea and blood in stool and sacrificed 7 days after the induction of colitis. Colitis was induced in Rag-1/- mice using CD45RBR1g2 lymphocytes from WT or A2BR-/- mice. Mice were followed for 70 days or until the development of weight loss at which time they were sacrificed. Clinical and immunologic scores were obtained according to established criteria. Results and Conclusions: WT mice administered with WT bone marrow (WT-WT) showed severe colitis (clinical score: 9.2 ± 0.2, histological score: 10 ± 1). In contrast, the severity of colitis was significantly reduced in A2BR-/- or A2BR+/- (clinical score: 6.0 ± 1.3 histological score: 4.2 ± 0.2) or WT-A2BR+/- mice (clinical score: 2.0 ± 0.6). Transfer of CD45RBR1g2 lymphocytes from WT or A2BR-/- mice to Rag-1/- mice resulted in significantly lower histological score compared to WT CD45RBR1g2 to Rag-1/- mice. This was reflected in significantly lower levels of colonic TNFα, KC and INF-γ levels in these mice. Conclusions: These data indicate that A2BR deletion on non-immune cells, possibly colonic epithelial cells, confers protection from colitis. We conclude that A2BR activation in the colonic mucosa drives inflammation and that mucosal A2BR is a potential target in the development of inflammatory bowel disease.

M1830

Deficiency of IL-17 Leads to the Suppression of Indomethacin-Induced Enteritis in Mice

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Background and Aims: Nonsteroidal anti-inflammatory drugs (NSAIDs) use is associated with an elevated risk of gastrointestinal damage. Especially, recent endoscopic technology revealed ER stress response mechanisms under conditions of chronic ileitis. To further elucidate the role of ER stress responses in the context of chronic ileitis using TNFα/ARE/WT mice. Methods & Results: Western blot analysis of primary ileal epithelial cells from TNFα/ARE/WT mice revealed ER stress response mechanisms under conditions of chronic ileitis. To further elucidate the role of TNFα and PFA in the regulation of ER stress, the small intestinal epithelial cell line Mode K was stimulated with the ER stressors tunicamycin and thapsigargin. Western blot analysis revealed that the induction of glucose regulated protein (GRP) 78 and C/EBP homologous protein (CHOP) was abrogated in the presence of TNFα and PFA. Although TNFα and PFA were supposed to act as a chaperones both failed to inhibit ER stress triggered phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α), suggesting that the accumulation of proteins in the ER is not affected. PFA and butyrate, both inhibitors of histone acetylation, transiently induced gene expression priming the epithelial cells towards an alleviated response upon subsequent ER stress induction. TUDCA in contrast does not alter histone acetylation in epithelial cells. Structure function analysis revealed that the endobiotic bile acid ursodeoxycholic acid (UDCA) and not taurine mediates the inhibitory effect of TUDCA. Chromatin immunoprecipitation (ChIP) analysis showed that TUDCA is able to inhibit the binding of the ER stress activated transcription factors (ATF) 4 and 6 as well as X-box binding protein (XBP1) to the grp78 promoter. Interestingly, repeated intraperitoneal (i.p.) injection of TUDCA or PFA did not alter ileal tissue pathology in TNFα/ARE/WT mice. However, western blot analysis of the lysed ileal explants from TNFα/ARE/WT mice revealed an abrogation of GRP78 expression by TUDCA, suggesting that i.p. administered chaperones may not reach the site of inflammation. Conclusions: These findings point out the pharmacological potential of the conjugated bile acid TUDCA and the short chain fatty acid derivative PFA in counteracting chronic intestinal inflammation, since these compounds attenuate ER stress responses in the epithelium, a key factor in the pathogenesis of IBD.

M1827

Gene Expression Profiling in the Small Intestine of Rats After the Indomethacin Exposure: Role of Matrix Metalloproteinases and Chemokines

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Background and Aims: Nonsteroidal anti-inflammatory drugs (NSAIDs) use is associated with an elevated risk of gastrointestinal damage. Especially, recent endoscopic technology has revealed that small intestinal injury is a serious complication in patients with IBD. However, the precise pathogenic mechanism of NSAIDs-induced small intestinal injury is still unknown. The aim of the present study was to analyze gene expression in rat intestinal tissue exposed to indomethacin using high-density oligonucleotide array, and to investigate the effects of rehmannia, a gastro-protective and anti-inflammatory drug, on the expression of these genes. Material and Methods: Intestinal injury was induced in male Wistar rats by intrarectal injection of indomethacin (15mg/kg) subcutaneously administration. Total RNA of the intestinal mucosa was extracted 24 h after indomethacin administration. Gene expression analysis was performed using GeneChip array (Affymetrix), and identified genes were confirmed by real-time PCR. Furthermore, we investigated whether moderate treatment with rehmannia improved these identified genes expression. Results: 1) The administration of indomethacin induced small intestinal injuries, and these lesions were significantly inhibited by the treatment with rehmannia. 2) The administration of indomethacin up-regulated the several matrix metalloproteinases (MMPs) gene and chemokine-related genes (CCL2 etc.) 3) These up-regulated genes were reversed by the treatment with rehmannia. Conclusion: These results indicate that MMPs and chemokines plays an important role in the intestinal mucosal injury induced by indomethacin. Furthermore, rehmannia inhibited indomethacin-induced intestinal injury by suppression of MMPs genes and chemokine-related genes. AGA Abstracts S-427