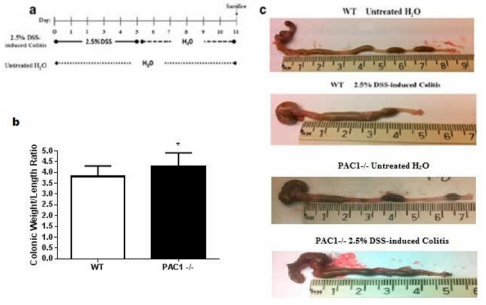
of PACAP are attributed to the expression of its specific receptor, PAC1, in a DSS model of colitis. **Aim:** We examined mice deficient in the PAC1 receptor to establish the role of the PACAP-PAC1 receptor axis in regulating the inflammatory response in DSS induced colitis. **Methods:** A total of 14 PAC1-/- mice and 16 Wild Type (WT) C57BL/129SV mice were given 2.5% DSS-treated water for 5 days. The mice were allowed 6 days of recovery with normal drinking water and sacrificed on day 11. Daily weights, water and food intake, as well as physical activity were measured throughout the study. Stool appearance was also observed. Upon euthanasia, intact colons from both groups of mice were extracted. To verify the extent of colitis, the colonic weight to length ratio was determined. Colonic tissue was examined histologically by H&E staining and scored in a blinded fashion. **Results:** PAC1-/- mice treated with DSS demonstrated a higher colonic weight to length ratio (4.23 ± 0.15), p = 0.004, indicating worsened colitis, compared to WT mice (3.57 ± 0.15). The mortality rate of the PAC1-/- mice was 50% versus 12.5% in the WT group. H&E staining of colonic tissue indicated that inflammation was augmented in the PAC1-/- mice compared to WT mice. **Conclusions:** PAC1 receptor deficient mice exposed to DSS showed a significantly higher mortality rate and developed more severe colitis with higher levels of colonic inflammation than wild type mice. This is in agreement with prior studies on PACAP -/- mice and suggests that the anti-inflammatory role of PACAP is mediated through activation of its specific receptor, PAC1.



Tu1886

Secreted Protein Acidic and Rich in Cysteine (SPARC) Contributes to Induce IL-17A Producing CD4$^+$ T Cell in Intestinal Inflammation

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Background: Secreted protein acidic and rich in cysteine (SPARC) plays a role in tissue remodeling and wound repair. However, the role of SPARC in intestinal inflammation has not been investigated. The present study aimed the association between SPARC and intestinal inflammation using a mouse experimental colitis model. **Methods:** C57BL/6 male SPARC wild-type and knock-out mice (SPARC$^{-/-}$) were received an enema of TNBS (2,4,6- trinitrobenezene sulfonic acid) to induce colitis. Distal colon was removed and assessed for colonic damage, histological score and MPO (Myeloperoxidase) activity. IL-17 mRNA expression were assessed by real-time PCR. LPLs (lamina propria lymphocytes) and lymphocytes in MLN (mesenteric lymph nodes) were analyzed by flow cytometry. **Results:** SPARC$^{-/-}$ mice exposed to TNBS showed less body weight loss and decreased histological inflammation compared to WT mice exposed to TNBS. MPO activity and IL-17A mRNA expression in the colonic mucosa were significantly higher($p<0.01$, $p<0.01$ respectively) in WT mice exposed to TNBS. However, MPO activity and cytokine expression were significantly inhibited($p<0.01$, $p<0.01$ respectively) in SPARC$^{-/-}$ mice exposed to TNBS. Furthermore, the percentages of IL-17A producing CD4$^+$ T cells were inhibited($p<0.01$)in SPARC$^{-/-}$ mice, compared to WT mice. **Conclusion**:In the present study, we determined the important role of SPARC to induce IL-17A producing CD4$^+$ T cells in TNBS-induced colitis model. Based on the results, SPARC could become a therapeutic molecular target for intestinal inflammatory disorders such as inflammatory bowel disease.

Tu1887

Revised Roles of Matrix Metalloproteinase/MMP-9 in Inflammatory Bowel Diseases/IBD: From Target to Biomarker

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Background: MMP-9 is elevated in blood and intestinal tissue of IBD patients and is suggested by knock-out (KO) mouse and inhibitor studies as a key causal factor. MMP-9 antagonists are presently evaluated in clinical trials for IBD. Our aim was to re-investigate the role of MMP-9 in acute and chronic intestinal inflammation. **Methods**: MMP-9 KO mice were backcrossed for 13 generations into C57BL/6J mice and bred under identical environmental conditions for more than 15 years in our animal facility. In 8-10 weeks old MMP-9 KO mice and their WT littermates, acute colitis was induced by oral administration of 3% dextran sodium sulphate (DSS) for 7 days followed by 2 days of regular water. Chronic colitis was induced by 3 cycles of 1 week 1.75-2.0% DSS each followed by a recovery phase of 2 weeks. Intestinal inflammation and fibrosis were assessed by macroscopic parameters, histopathology analysis and tissue collagen levels. In colonic tissue, MMP-9 levels were determined by gelatin zymography analysis and gene expression differences were assessed with RNA sequencing (Illumina HiSeq2500, fold change [FC]>2, 10% false discovery rate [FDR]). Pharmacological inhibition of MMP-9 was tested by administration of two peptide inhibitors (CPU1 and CPU2) to acute DSS-treated C57BL/6J mice via daily intraperitoneal injections (250 µg) and via implanted osmotic pumps (30 mg/kg/day). The inhibitory effect was evaluated by clinical, histopathological and qRT-PCR analyses. **Results**: In contrast to previously reported phenotypes, clinical and histopathological parameters were not attenuated in MMP-9 KO mice after acute DSS administration compared with WT littermates. Zymography analysis confirmed absence of MMP-9 in our KO mice and showed increased MMP-9 levels after DSS in WT mice only. Similar expression of host genes in WT and MMP-9 KO control mice was observed, with exception of *Mmp9* (FC=3.8), *Rims4* (FC=-6.0) and *Slpi* (FC=2.5). After induction of colitis, 11 genes involved in antimicrobial response were differentially expressed between WT and MMP-9 KO mice. Development of fibrosis was not altered in chronic DSS-treated MMP-9 KO mice, although less goblet cell loss was observed compared with WT mice. Pharmacological inhibition of MMP-9 with CPU1 and CPU2 did not improve parameters of intestinal inflammation. On the contrary, increased *Mmp3, Mmp8* and *Mmp9* expression was observed in DSS-treated mice that received CPU2 compared with saline. **Conclusions**: Against prevailing evidences, we demonstrate that MMP-9 deletion or inhibition does not lead to clinical and histopathological attenuation of DSS-induced colitis. Whereas MMP-9 remains an excellent inflammatory marker in IBD, ongoing clinical trials with MMP-9 inhibition as a therapeutic option for IBD need to be carefully followed.

Tu1888

Deficiency of CCR7 Exacerbates Non-Steroidal Anti-Inflammatory Drug-Induced Enteropathy in Mice

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Background/Aim: Incident of adverse events by non-steroidal anti-inflammatory drug (NSAID) on stomach and duodenum have been well reported. Recent technological development including video capsule endoscopy and balloon assisted endoscopy revealed that patients who continuously use NSAID often have mucosal damages in the small intestine. Although the pathogenesis of NSAID-induced enteropathy has been suggested to be multifactorial, such as changes in mucosal permeability, bile acid, proteolytic enzyme, intestinal bacteria, and inflammatory cytokines, it has not been completely elucidated. The chemokine receptor CCR7 mediates mucosal immune homeostasis by regulating migration of lymphocytes as well as dendritic cells to and within lymphoid organs. In this study, we aimed to clarify the role of CCR7 in NSAID-induced enteropathy. Method: Male CCR7-deficient (CCR7$^{-/-}$, C57BL/6 background, 8-9 weeks of age) mice and counterpart wild-type (WT) mice were used in this study. All mice were kept under specific pathogen free conditions. Enteropathy was induced by subcutaneous injection with indomethacin (10.0 mg/kg body weight). Twenty-four hours after the injection with indomethacin, the mice were analyzed. The tissues of the small intestine were fixed with 4% paraformaldehyde, and the ulcer area was measured by tracing the ulcers in the small intestine under a stereomicroscope. Mononuclear cells isolated from the mesenteric lymph nodes (MLN) and small intestinal lamina propria (LP) were isolated and cellular profiles were analyzed by flow cytometry. Results: Both WT and CCR7$^{-/-}$ mice showed multiple ulcers in the small intestine after subcutaneous injection of indomethacin, whereas both of them did not spontaneously exhibit mucosal inflammation in the small intestine. CCR7$^{-/-}$ mice exhibited wider area of ulceration compared to WT mice after indomethacin injection. Total number of mononuclear cells in MLN was significantly lower in CCR7$^{-/-}$ mice than in WT mice. The proportion of CD8$^+$ cells was significantly higher in the MLN, but it was significantly lower in LP of CCR7$^{-/-}$ mice than WT mice. The number of CD11c$^+$CD103$^+$ cells, which have been shown to have an ability to induce IL-10-producing Tr1 cells, were significantly lower in CCR7$^{-/-}$ mice than WT mice in MLN and LP. The proportion of CD4$^+$Foxp3$^+$ Treg cells in the MLN was significantly higher in CCR7$^{-/-}$ mice than in WT mice. Conclusions: Recruitment of immune cells such as CD11c$^+$CD103$^+$ cells via CCR7 may contribute to the mucosal protection in NSAID-induced enteropathy.

Tu1889

Targeting of NLRP3 Inflammasome With a Novel Selective Inhibitor as a Suitable Strategy for the Pharmacological Treatment of Bowel Inflammation

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Introduction. NLRP3 inflammasome, a protein complex responsible for the proteolytic maturation and secretion of the pro-inflammatory cytokines IL-1beta and IL-18, has been shown to regulate the integrity of intestinal mucosal barrier and play a pivotal role in shaping the immune response against commensal microbiota during bowel inflammation. However, there are currently few selective drug candidates suitable for treatment of intestinal inflammation through the modulation of inflammasomes. This study examined the effects of a novel selective NLRP3 inflammasome inhibitor in an experimental model of colitis. **Methods.** The effects of INF39E (novel selective NLRP3 inflammasome inhibitor) and dexamethasone (DEX, used as a standard comparator) were tested in male rats (n=6 for each group) with colitis induced by intrarectal administration of 2,4-dinitrobenzenesulfonic acid (DNBS, 15 mg/rat), to assess systemic [body and spleen weight] and tissue inflammatory parameters [macroscopic, tumor necrosis factor (TNF), interleukin-1beta (IL-1beta), and myeloperoxidase (MPO) levels]. Animals received INF39E (12.5, 25, 50 mg/kg/day), DEX (1 mg/kg/day) or vehicle orally for 6 days, starting the same day of DNBS administration. **Results.** Colitis was associated with a decrease in body weight and an increase in spleen weight. The macroscopic damage score, as well as tissue TNF, IL-1beta and MPO levels were increased. Treatment with INF39E, but not DEX, improved body weight. Both drugs counteracted the increase in spleen weight and ameliorated the macroscopic damage score. A significant reduction of IL-beta tissue levels was recorded in rats treated with INF39E 25 and 50 mg/kg/day or DEX. Moreover, INF39E 25 and 50 mg/kg/day or DEX ameliorated colonic MPO levels and decreased tissue TNF levels. The overall results concerning the effects of INF39E and DEX on the inflammatory parameters are displayed in the table. **Conclusions.** The novel NLRP3 inflammasome inhibitor exerts beneficial effects on bowel inflammation, through a reduction of pro-inflammatory cytokine levels, mainly IL-1beta. These findings substantiate the concept that the pharmacological modulation of the NLRP3 inflammasome complex represents a promising strategy to develop novel classes of drugs effective against intestinal inflammation.

Summary of the results obtained in the study

	Body weight changes (%)	Spleen weight change (%)	Macroscopic damage score	TNF (pg/mg tissue)	IL-1beta (pg/mg tissue)	MPO (ng/mg tissue)
Control	+20	0	1.8	2.6	9.8	3.9
DNBS	-63*	+31.6*	8.7*	5.9*	20.9*	48.1*
INF39E 12.5 mg/Kg	+19**	+2**	5.1**	3.4	15.8**	15.2**
INF39E 25 mg/Kg	+22**	+4**	1.9**	3.1**	10.8**	18.8**
INF39E 50 mg/Kg	+21**	+10**	2**	2.9**	8.1**	13.3**
DEX 1mg/Kg	-75 *, **	-14**	4.7**	2.2**	6.7**	15.6**

*P<0.05 vs control and **P<0.05 vs DNBS, ANOVA. Abbreviations: TNF, tumor necrosis factor; IL-1beta, interleukin-1beta; MPO: myeloperoxidase.

Tu1890

Refinement of the *S. Typhimurium* Model of Colitis for Anti-Fibrotic Drug Discovery and Pre-Clinical Applications

Laura A. Johnson, Eva S. Rodansky, Peter Higgins

Background: Intestinal fibrosis in Crohn's disease (CD) is a classic example of organ fibrosis, in which recurring cycles of inflammation and mucosal healing ultimately cumulate in irreversible organ damage, intestinal blockage, and surgical removal of intestine in a majority of patients. The mouse *Salmonella typhimurium* model of colitis and fibrosis, due to its simplicity, reproducibility, and efficacy is an attractive model for drug discovery and pre-clinical trials. However, both mortality and fibrotic disease are host-dependent. **Aims:** To optimize intestinal fibrosis in the mouse *S. typhimurium* model using alternative, commercially available mouse strains. **Methods:** Three mouse strains (129S1/SvImJ, DBA/J, and CBA/J) were infected with either SL1344 or ΔaroA *S. typhimurium* strains. Survival and extent of fibrosis was determined by histopathology, pro-fibrotic gene expression and αSMA protein expression. **Results:** 100% survival was observed in all three strains infected with ΔaroA. Notable strain-specific mortality occurred with SL1344 infection; DBA/J, 100% at day 7; 129S1/SvImJ, 60% at day 14. In contrast, 100% survival and marked tissue fibrosis occurred in the CBA/J strain infected with SL1344 as determined by histopathology and αSMA protein expression. Subsequent experiments demonstrated that CBA/J mice develop extensive intestinal fibrosis, characterized by transmural tissue fibrosis, a Th1/Th17 cytokine response, and induction of profibrotic genes and ECM proteins. **Conclusion(s):** The CBA/J mice infected with wild-type SL1344 *S. typhimurium* results in highly penetrant intestinal fibrosis with 100% survival in the mouse *S. typhimurium* model of intestinal fibrosis.

Tu1891

Anti-Inflammatory Effects and Mechanisms of Vagal Nerve Stimulation Combined With Electroacupuncture in a Rodent Model of TNBS-Induced Colitis

Haifeng Jin, Jie Guo, Jiemin Liu, Lu Bin, Robert Foreman, Jieyun Yin, Zhaohong Shi, Jiande Chen

Background/Aims: Vagal nerve stimulation (VNS) has been shown to exert an anti-inflammatory effect; electroacupuncture (EA) is also known to have an anti-inflammatory effect. The aim of this study was to determine effects and mechanisms of combined VNS and EA on intestinal inflammation in a rodent model of ulcerative colitis. **Methods** Chronic inflammation in rats was induced by intrarectal TNBS (2,4,6-Trinitrobenzenesulfonic acid). The rats were then treated with sham-ES (electrical stimulation), VNS or VNS+EA for 3 weeks. Inflammatory responses were assessed by disease activity index (DAI), macroscopic scores and histological scores of colonic tissues, and plasma levels of TNF-α, IL-1β and IL-6, myeloperoxidase (MPO) activity of colonic tissues. The autonomic function was assessed by the spectral analysis of the heart rate variability (HRV) derived from the electrocardiogram. **Results** 1) The area under curve (AUC) of DAI during the entire study was substantially decreased with VNS+EA and VNS, compared to the sham-ES group (P<0.001 for both), and VNS+EA was more effective than VNS (P<0.001); 2) The macroscopic score was 6.43±0.61 in the sham-ES group and reduced to 1.86±0.26 with VNS (P<0.001) and 1.29±0.18 with VNS+EA (P<0.001). The histological score was 4.05±0.58 in the sham-ES group and reduced to 1.93±0.37 with VNS (P<0.001) and 1.36±0.20 with VNS+EA (P<0.001); 4) the plasma levels of TNF-α, IL-1β, IL-6 and MPO were all significantly decreased with VNS and VNS+EA, compared to the sham-ES group; 5) Neurally, both VNS+EA and VNS substantially increased vagal activity and decreased sympathetic activity in comparison with sham-EA group (P<0.001, P<0.001, respectively). **Conclusions:** Chronic VNS improves inflammation in TNBS-treated rats by inhibiting pro-inflammatory cytokines via the autonomic mechanism. Addition of noninvasive EA to VNS significantly enhances the anti-inflammatory effect of VNS.

Tu1892

Electrical Stimulation via Chronically Implanted Electrodes at Acupoints Improves TNBS-induced Colonic Inflammation via Autonomic Mechanism in Rats

Haifeng Jin, Jie Guo, Robert Foreman, Lu Bin, Zhaohong Shi, Jieyun Yin, Jiande Chen

Introduction Chronic inflammation in inflammatory bowel diseases (IBD) is hypothesized to be at least partially attributed to an imbalance between sympathetic and parasympathetic activities. Electroacupuncture (EA) has been shown to improve sympathovagal balance. The purpose of this study was to determine possible effects and mechanisms of electroacupuncture (EA) via chronically implanted electrodes at ST36 on TNBS-induced colonic inflammation. **Methods** Colitis in rats was induced by intrarectal administration of 2,4,6-Trinitrobenzenesulfonic acid (TNBS). The rats were treated with sham-EA, EA1 (EA using parameters of: 25Hz, 2s on, 3s off, 0.5ms, 4.0 mA) or EA2 (EA using parameters of: 5Hz, 10s on, 90s off, 0.5ms, 4.0mA) for 3 weeks. A control group (received rectal injection of saline) was also followed for 3 weeks. Disease activity index (DAI), macroscopic and microscopic lesions, plasma levels of inflammatory cytokines (TNF-α, IL-1β and IL-6) and myeloperoxidase (MPO) activity of colonic tissues were assessed. The autonomic function was assessed by the spectral analysis of the heart rate variability (HRV) derived from the electrocardiogram. **Results** 1) The vagal activity was significantly increased with acute EA1 and EA2 both during and 30-min after EA; 2) DAI in the TNBS-treated rats was significantly decreased by EA1 and EA2, compared to the sham-EA group (P<0.05, P<0.01, respectively), and EA2 was more effective than EA1 (P<0.05); 3) The macroscopic score was 6.43±0.61 in the sham-EA group and reduced to 4.86±0.14 with EA1 (P<0.05) and 4.0 ±0.22 with EA2 (P<0.001); EA2 was more effective than EA1 (P=0.017). The histological score was 4.05±0.58 in the sham-EA group and reduced to 3.71±0.28 with EA1 (P>0.05) and 3.0±0.28 with EA2 (P<0.01). The MPO activity of colonic tissue was also significantly reduced with both EA1 and EA2; 4) the plasma levels of TNF-α, IL-1β and IL-6 were all significantly decreased with EA1 and EA2, compared to the sham-EA group; 5) Autonomically, both chronic EA1 and EA2 significantly increased vagal activity and decreased sympathetic activity in comparison with sham-EA group. **Conclusions** Chronic EA using chronically implanted electrodes improves colonic inflammation in TNBS-treated rats by inhibiting pro-inflammatory cytokines via the autonomic mechanism.

Tu1893

Human Catestatin Represses Reactivation of Intestinal Inflammation in a Murine Model of Colitis Through the M1 Macrophages and Not the Gut Microbiota

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Background: While there is growing awareness of a relationship between chromogranin-A and a susceptibility to inflammatory conditions, the role of catestatin (CTS; human (h) chromogranin A $(CgA)_{352-67}$) in the natural history of established inflammatory bowel disease (IBD) is not known. Recently, using 2 different models, we have shown that hCTS-treated mice develop a less severe acute experimental colitis compared to controls. We have also shown that the macrophages are involved in this effect. The aims of this study were to determine (1) whether a hCTS treatment could attenuate the reactivation of inflammation in adult mice with previously established chronic colitis; (2) whether this effect was mediated trough the macrophages, the apoptotic pathway or the gut microbiota. **Approach:** Chronic relapsing colitis was induced in 7-8 weeks C57BL6 mice using 4 cycles dextran sulfate sodium (DSS) (4%, 2%, 2%, 4%, 5 days each followed by 11 days rest). Preventive hCTS (1.5 mg/kg/day) treatment or vehicle started 2 days before the last induction and lasted for 7 days. Weight loss was determined daily. At sacrifice, macro- and microscopic scores, colonic pro-inflammatory cytokines (IL-6, IL-1β and TNF-α), classical activated (M1) (iNOS, MCP-1), alternatively activated (M2) (YM1, FIZZ1, ARG-1) macrophages markers, and intrinsic pro-apoptotic markers (PUMA, BAX, BAD, BAK1) were determined using ELISA and/or RT-qPCR. *In-vitro*, peritoneal macrophages isolated from naïve mice and treated with hCTS (10^{-5}M) were exposed to LPS (100ng/ml) for 12h to polarize M1 macrophages and proinflammatory cytokine levels were determined in supernatant. Feces and mucosa associated microbiota (MAM) samples were collected and the V4 region of 16s rRNA was subjected to Miseq Illumina sequencing. **Results:** hCTS therapy significantly reduced the vulnerability to DSS 4% reactivation. Micro- and macroscopic scores, colonic IL-6, IL-1 β, TNFα and M1 macrophages markers (iNOS, MCP-1) were significantly decreased in hCTS treated group. *In vitro,* pro-inflammatory cytokines levels were significantly reduced in hCTS-treated M1 macrophages. hCTS treatment regulated the colonic intrinsic apoptotic pathway through PUMA deactivation without affecting its downstream signals (BAD, BAX, BAK1). In colitic fecal and MAM samples, hCTS treatment did not modify the bacterial richness (alpha diversity) or diversity (beta diversity). Moreover, the treatment did not demonstrate a significant effect at the phylum or genus levels in both fecal and MAM samples. **Conclusion:** These findings suggest that hCTS treatment could attenuate the severity of the inflammatory relapse through the modulation of the M1 macrophages, proinflammatory cytokines and the colonic intrinsic apoptotic pathway wiht no effect on the gut microbiota. This study might open a new therapeutic avenue to treat chronic intestinal inflammation.

Tu1894

Schistosoma Mansoni Coinfection attenuates Murine Toxoplasma Gondii-Induced Crohn's-Like Ileitis by Modulating the Immune Response and the Number of Paneth Cells

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Background and aims: Orally T.gondii-infected mice develop Crohn's disease (CD)-like enteritis associated with severe systemic inflammatory response, and resultant increased morbidity and mortality. Previous data have shown that S.mansoni and other helminthic infections may have a therapeutic potential in experimental colitis. However, the role of