



HAL
open science

Par-2-1-induced Inflammation of Mouse Colon Involves Afferent Neurons and Nitric Oxide but Does Not Result from Increased Paracellular Permeability

Nicolas Cénac, Rafael Garcia Villar, Jean Fioramonti, Lionel Bueno

► **To cite this version:**

Nicolas Cénac, Rafael Garcia Villar, Jean Fioramonti, Lionel Bueno. Par-2-1-induced Inflammation of Mouse Colon Involves Afferent Neurons and Nitric Oxide but Does Not Result from Increased Paracellular Permeability. Digestive Disease Week / 103rd Annual meeting of the AGA, May 2002, San Francisco, California, United States. 2002, Gastroenterology 122 (Suppl 4). hal-01606335

HAL Id: hal-01606335

<https://hal.science/hal-01606335>

Submitted on 4 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

S958

The Roles of TGF β and COX-2 in the Maintenance of Muscle Hypercontractility in a Murine Model of Post-Infective Irritable Bowel Syndrome

Hirotada Akiho, Yikang Deng, Patricia Blennerhassett, Hiroshi Kanbayashi, Giovanni Barbara, Robert Degiorgio, Stephen Collins, Hamilton, ON, Canada; Bologna, Italy

Background/Aims: Acute gastroenteritis is a strong risk factor for the development of Irritable Bowel Syndrome (IBS). We have developed an animal model in which transient acute infection leads to persistent intestinal muscle hypercontractility which can be reversed using COX inhibitors (Barbara et al Gastroenterology 1997, 2001). Putative mediators of this response include IL-4, IL-13 or TGF β 1. Recently, we showed that this hypercontractility is evident in muscle cells isolated from the gut 35 days post-infection (Akiho et al DDW 2001). The aim of this study was to investigate the relationship between the above described cytokines and COX-2. **Methods:** mRNA or protein expression of cytokines or inflammatory mediators were examined from jejunal longitudinal muscle myenteric plexus of NIH Swiss mice infected with or without *Trichinella spiralis*, or from primary cultured longitudinal muscle cells of mice incubated with or without cytokines. **Results:** During the acute phase of infection there was significantly increased expression of Th2 cytokines, IL-4 or IL-13, TGF β 1 and COX-2 mRNA in the muscle. Post infection, expression of Th2 cytokines returned to normal, but TGF β 1 and COX-2 mRNA remained elevated in the muscle ($p < 0.05$). Exposure of cultured muscle cells to 100ng/ml of IL-4 or 100ng/ml of IL-13 increased TGF β 1 ($p < 0.01$) and PGE $_2$ ($p < 0.05$) protein expression. Exposure of cultured muscle cells to 100ng/ml of TGF β 1 increased COX-2 and PGE $_2$ ($p < 0.05$) protein expression. **Conclusions:** These results describe a model in which Th2 cytokines initiate muscle hypercontractility during the acute infection but in which the changes are maintained post-infection by the presence of TGF β 1 in the muscularis externa. TGF β 1 induces COX-2 and promotes PGE $_2$ production at the level of the muscle cell, thus maintaining hypercontractility. These results open new therapeutic avenues for treating IBS. Supported by Canadian Institutes for Health Research and Astra Zeneca.

S959

Identification of a Selective Agonist for the Orphan Receptor, BRS-3

Samuel A. Mantey, Laurence Entsuah, David H. Coy, Jeremiah Ojeburu, Hisato Igarashi, Carmen Mateo, Tomoo Nakagawa, Lin Shen, Robert T. Jensen, Bethesda, MD; New Orleans, LA

BKG: The orphan receptor, BRS-3 is homologous (50%) with mammalian bombesin (Bn) receptors, gastrin-releasing peptide receptor (GRPR) and neuromedin B receptor (NMBR). Its natural ligand is unknown but BRS-3 is widely distributed in the CNS and GI tract. Targeted disruption of BRS-3 leads to obesity, diabetes and hypertension. However, its role in physiological and pathological processes remain unknown because there is no sufficiently selective agonists or antagonists to be useful. In previous studies we discovered a synthetic Bn analogue, (DTyr⁶, β Ala¹¹,Phe¹³,Nle¹⁴)Bn(6-14) with high affinity for BRS-3. The use of this ligand is, however, limited because of its high affinity for all the known Bn receptors. Recently we found that substitution at the β Ala¹¹ position with conformationally restricted amino acids, (R) and (S)3-amino 3-phenylpropionic acid (Apa) yielded a peptide that had low (8-10) fold selectivity for the BRS-3 over the GRPR/NMBR. **AIM:** The aim of this study was to design peptides more selective for the BRS-3. **METHODS:** We used as our reference peptide, (DTyr⁶,Apa¹¹,Phe¹³,Nle¹⁴)Bn(6-14) and synthesized 11 peptides with alterations at the Apa in position 11 ((S)Apa-2Cl, (R)Apa-2Cl, (S)Apa-4Cl, (R)Apa-4Cl, (S)Apa-3-Cl, (R)Apa-3-Cl, β Asp, β Asp(BzIAMD), β Dap, N α -benzoyl-Dap, N α -acetyl-Dap). Using BALB 3T3 cells stably transfected with the 3 human Bn receptor subtypes (hBRS-3, hNMBR and hGRPR) we determined their binding affinities and biological activities. **RESULTS:** Of the 11 new peptides, (DTyr⁶,(R)Apa¹¹-4Cl,Phe¹³,Nle¹⁴)Bn(6-14) showed enhanced selectivity for BRS-3. Its affinity for BRS-3 ($K_d = 8$ nM) was 230-fold higher than for GRPR and 880-fold higher than NMBR. This analogue was a full agonist and its selectivity was confirmed in biological assays assessing changes in [3H]IP. This analogue did not interact with CCK $_R$, CCK $_B$, NK1, M3 muscarinic receptors or somatostatin receptors. **CONCLUSIONS:** The availability of this selective agonist for the BRS-3 receptor should prove useful in elucidating the physiology and pathophysiology of this orphan receptor in body weight, blood pressure and blood sugar regulation.

S960

On the Relationship of IBS and IBD: Estrogen Induces a Sustained Increase in Colonic Substance P Levels Post DNB Colitis.

Elena F. Verdu, Alexandra Bongard, Premysl Bercik, Yikang Deng, Stephen M. Collins, Hamilton, Canada

Background: Inflammatory Bowel Disease (IBD) may be a risk factor for the development of Irritable Bowel Syndrome (IBS) in some individuals (Isgar et al. Gut 1983). IBS is more common in females and may reflect effects of reproductive hormones on gut function. Hyperalgesia is a common finding in IBS and may be mediated via the sensory transmitter Substance P (SP). In this study, we examined the relationship of colitis and SP levels in mice with or without 17 β -estradiol (E2) pre-treatment. **Methods:** Colitis was induced in female and male C57BL/6 mice (n=5-7 per group) by intra-rectal administration of dinitro-benzene sulfonic acid (DNB) in 50% ethanol and assessed macroscopically, histologically and by myeloperoxidase (MPO) activity. Mice without colitis were used as controls. Vaginal swabs determined the phase of estrous cycle at colitis induction. Additional female mice were implanted subcutaneously with 21 day-release pellets containing E2 (0.5mg/pellet) or placebo. Mice were euthanized 3 (acute) 7 and 14 (recovery) days after colitis induction. SP was measured by Enzyme Immuno Assay in colonic tissue adjacent to the ulcer on days 3 and 7 post-colitis, and mRNA for SP was measured on day 14 post-colitis by RT-PCR. **Results:** There were no gender differences in macroscopic and histologic scores, MPO activity or SP levels 3 days post DNB colitis. Colitis induced a 2.5 fold increase in SP but no gender differences were seen. Although colitis induced during the high estrogen phase of the cycle did not affect the severity of colitis or SP levels, pre-treatment with E2 ameliorated colitis and produced a smaller increment in SP than in placebo-treated mice with colitis, 24.6 ng/ml (± 5.2) vs 29.6 ng/ml (± 5.0) respectively. However, while SP levels returned to normal

(16.5 ng/ml ± 5.6) in placebo-treated mice 7 days post colitis, values for SP increased to 46.0 ng/ml (± 23.9) in E2 treated mice. Moreover, at day 14 post-colitis SP/G3PDH mRNA ratio was 2.5-fold higher in E2-treated mice than in placebo-treated mice. **Conclusions:** These results indicate that estrogen treatment induces a significant and persistent increase in colonic SP evident at least 2 weeks post colitis. It is possible that the risk of IBS post IBD may be higher in patients receiving estrogen supplements. Supported by Canadian Institutes of Health Research.

S961

Immunoregulation of Somatostatin, Gastrin and Substance P by IFN γ and IL-4 in the Mouse Gastric Mucosa.

Yana Zavros, Joel V. Weinstock, Juanita L. Merchant, Ann Arbor, MI; Iowa City, IA

Background: Patients infected with *Helicobacter pylori* (*H. pylori*) have increased IFN γ -expressing T lymphocytes (Th1 immune response). Gastritis causes reciprocal changes in the neuroendocrine cell populations, gastrin (G-) and somatostatin (SOM)-secreting D-cells. Therefore, first we queried whether the proinflammatory cytokine IFN γ alone was sufficient to cause the perturbations observed in the neuroendocrine cells independent of the bacteria. SOM and substance P (SP) are short polypeptides that can mediate the expression of the Th1 immune response. While SP stimulates, SOM inhibits IFN γ expression. The Th2 specific cytokine, IL-4, is believed to antagonize the Th1 immune response in part by inhibiting the release of SP. The role of SOM as a mediator of IL-4 suppression on IFN γ is less clear. Therefore, we studied the regulation of SOM and SP by IFN γ and IL-4 *in vivo*. **Methods:** PBS, IFN γ or IL-4 was infused into C57BL/6 mice for 7 days. Plasma gastrin concentrations and tissue SOM content were measured by radioimmunoassay. Tissue sections were fixed for histology and morphometric analysis and immunostained for SOM and gastrin. SP peptide levels were quantified by western blot analysis. Sections were stained for H&E and PAS/alcian blue and graded on the intensity of gastritis. **Results:** After IFN γ infusion, tissue SOM content decreased by 50%; whereas, the number of G-cells and plasma gastrin increased 3-fold. In addition, SP protein expression increased significantly by 5-fold. Histologic evaluation of the gastric mucosa revealed the presence of lymphocytes, leukocytes and mucous gland metaplasia after 7 days of IFN γ infusion. In contrast, there were no inflammatory or metaplastic changes in the IL-4 treated mice. In addition, IL-4 significantly decreased gastrin concentrations (90%) and SP protein expression (50%). This correlated with a 2-fold increase in SOM. **Conclusions:** It is likely that the release of IFN γ from Th1 lymphocytes mediates the hypergastrinemia observed during *H. pylori* infection. The effect of IFN γ on the G cell may be indirect through the release of SP. In contrast, IL-4 inhibits gastrin release via stimulation of SOM and inhibition of SP.

S962

Par-2-Induced Inflammation of Mouse Colon Involves Afferent Neurons and Nitric Oxide but Does Not Result from Increased Paracellular Permeability

Nicolas Cenac, Rafael Garcia-Villar, Jean Fioramonti, Lionel Buéno, Toulouse, France

Activation of colonic PAR-2 receptors provoked inflammation and increased mucosal permeability in mice colon. The mechanism of this inflammation is under debate but evidence suggests that it could be neurogenic (NANC neurons) and/or result from the opening of colonic tight junctions (TJs) with subsequent passage of exogenous macromolecules into the lamina propria. We aimed to further characterize PAR-2-induced inflammatory effect by investigating the involvement of (i) nitric oxide, (ii) capsaicin-sensitive afferent neurons, and (iii) a possible cause and effect relationship between the enhancement of paracellular permeability and mucosal inflammation. **Methods: Thirteen groups of 8 male Swiss mice (26-31 g) were infused intracolonic (ic, 50 μ l) with either the PAR-2 activating peptide SLIGRL (100 μ g/mouse) or saline (controls), followed from 2 to 4 h later by 2.10⁸ cpm ⁵¹Cr-EDTA (750 μ l/h). Prior to these infusions (i) six groups received either the iNOS inhibitor aminoguanidine (1.5mg/mouse, IP), the NOS inhibitor L-NAME (0.6 mg/mouse, IP) or vehicle; (ii) four groups were treated with capsaicin (0.75 mg/mouse twice/day for 2 days) or vehicle; (iii) three groups received the TJ blocker TAP (2,4,6-triaminopyrimidine; 200 mM, ic) or vehicle. At 4 h blood was collected for radioactivity counting (cpm/ml) and colons for myeloperoxidase assay (MPO; units/g protein) and histologic examination (microscopic damage score, MDS). Values are the mean \pm SEM for each group. **Results:** In all treated groups, ic SLIGRL increased MPO, MDS and paracellular permeability values. (i) Both L-NAME and aminoguanidine prevented SLIGRL-induced increases in MPO (75.7 \pm 16.4 and 98.1 \pm 31.0 vs. 390 \pm 109), MDS (1.3 \pm 0.5 and 1.9 \pm 0.6 vs. 4.8 \pm 0.6) and permeability (556 \pm 108 and 783 \pm 95 vs. 1703 \pm 328). (ii) Similarly capsaicin prevented SLIGRL-induced increases in MPO (46.1 \pm 9.4 vs. 596 \pm 195), MDS (0.8 \pm 0.2 vs. 5.1 \pm 0.4) and permeability (666 \pm 125 vs. 1942 \pm 215). (iii) In contrast, although TAP prevented SLIGRL-induced permeability increase (119 \pm 56 vs. 676 \pm 95), it did not prevent increases in MPO (982 \pm 279 vs. 950 \pm 144) and MDS (4.9 \pm 0.3 vs. 5 \pm 0.8). **Conclusion:** Our data show that both nitric oxide and capsaicin-sensitive afferent neurons are involved in PAR-2-mediated colonic inflammation and increased paracellular permeability. Nevertheless the inflammation process does not appear to result from the observed increase of permeability.**

S963

Interaction of Glucagon-Like Peptide-2 and Common Therapeutics in a Murine Model of Ulcerative Colitis

Marie-Claude L'Heureux, Wendy Banfi, Patricia L. Brubaker, Toronto, ON, Canada

BACKGROUND: 5-aminosalicylates (ASA) and corticosteroids (CS) are effective agents for the relief of the symptoms associated with ulcerative colitis. In contrast, glucagon-like peptide-2 (GLP-2), a potent growth factor, has promising therapeutic properties because of its ability to stimulate intestinal growth and function. We have previously shown that GLP-2 enhances the reparative response to dextran sulfate (DS)-induced colitis in mice. We hypothesized that the intestinotropic effects of GLP-2 are enhanced by therapeutic agents commonly used to treat colitis. **METHODS:** Each therapeutic agent was administered alone or concomitantly with