Differential immune and genetic responses in rat models of Crohn’s colitis and ulcerative colitis

Xuan-Zheng Shi, John H. Winston, and Sushil K. Sarna

1Enteric Neuromuscular Disorders and Visceral Pain Center, Division of Gastroenterology, Department of Internal Medicine and 2Department of Neuroscience and Cell Biology, The University of Texas Medical Branch at Galveston, Galveston, Texas

Submitted 27 July 2010; accepted in final form 5 October 2010

Shi XZ, Winston JH, Sarna SK. Differential immune and genetic responses in rat models of Crohn’s colitis and ulcerative colitis. Am J Physiol Gastrointest Liver Physiol 300: G41–G51, 2011. First published October 14, 2010; doi:10.1152/ajpgi.00358.2010.—Crohn’s disease and ulcerative colitis are clinically, immunologically, and morphologically distinct forms of inflammatory bowel disease (IBD). However, smooth muscle function is impaired similarly in both diseases, resulting in diarrhea. We tested the hypothesis that differential cellular, genetic, and immunological mechanisms mediate smooth muscle dysfunction in two animal models believed to represent the two diseases. We used the rat models of trinitrobenzene sulfonic acid (TNBS)and dextran sodium sulfate (DSS)-induced colonic inflammations, which closely mimic the clinical and morphological features of Crohn’s disease and ulcerative colitis, respectively. DSS inflammation induced oxidative stress initially in mucosa/submucosa, which then propagated to the muscularis externa to impair smooth muscle function. The muscularis externa showed no increase of cytokines/chemokines. On the other hand, TNBS inflammation almost simultaneously induced oxidative stress, recruited or activated immune cells, and generated cytokines/chemokines in both mucosa/submucosa and muscularis externa. The generation of cytokines/chemokines did not correlate with the recruitment and activation of immune cells. Consequently, the impairment of smooth muscle function in DSS inflammation was primarily due to oxidative stress, whereas that in TNBS inflammation was due to both oxidative stress and proinflammatory cytokines. The impairment of smooth muscle function in DSS inflammation was due to suppression of Gaq protein of the excitation-contraction coupling. In TNBS inflammation, it was due to suppression of the α1Cβ1 subunit of Ca2+ channels, CPI-17 and Gα12. TNBS inflammation increased IGF-1 and TGF-β time dependently in the muscularis externa. IGF-1 induced smooth muscle hyperplasia; both IGF-1 and TGF-β induced hypertrophy. In conclusion, both TNBS and DSS induce transmural inflammation, albeit with different types of inflammatory mediators. The recruitment or activation of immune cells does not correlate directly with the intensity of generation of inflammatory mediators. The inflammatory mediators in TNBS and DSS inflammations target different genes to impair smooth muscle function.

inflammation, as defined by the infiltration of inflammatory white blood cells, in ulcerative colitis patients is superficial and limited mostly to the mucosa (32). We do not understand the etiologies of IBD; however, most evidence indicates that they might be different for the two forms of this disease. As a result, the treatment modalities of Crohn’s disease and ulcerative colitis might differ (34).

Despite differing etiologies, the primary symptoms of Crohn’s disease and ulcerative colitis, such as diarrhea, abdominal pain, and urgency of defecation, are similar. These symptoms result from the dysfunction of nonimmune cells in the gut wall, such as smooth muscle cells, enteric neurons, and epithelial cells that regulate the motility and transport functions. The ideal management of IBD is to prevent inflammation from relapsing. However, the current therapies of IBD using this approach have yielded limited success and are associated with adverse side effects, specifically in long-term use (10, 33, 34). An adjuvant approach would be to minimize the morbidity of this disease by targeting nonimmune cells to reduce or prevent their dysfunction and hence the symptoms of IBD.

Numerous studies have reported that despite the differences in etiologies and morphology of Crohn’s disease and ulcerative colitis, the circular smooth muscle contractions and tone are impaired in both (1, 44, 48, 50). This may account for the common symptom of diarrhea in both forms of IBD. The suppression of smooth muscle phasic contractions and tone contribute to diarrhea by suppressing the organized slow distal propulsion of fecal material and compromising its frequent turnover for maximal exposure to the absorptive mucosa (40). The impairment of smooth muscle function in ulcerative colitis raises the question whether the inflammation in ulcerative colitis is really just in the mucosa.

We used the rat models of Crohn’s disease and ulcerative colitis to investigate the differential immune and genetic responses that mediate circular smooth muscle dysfunction. We recognize that there is seldom a perfect model of human disease. Colonic inflammation induced by intraluminal infusion of the hapten trinitrobenzene sulfonic acid (TNBS) or oral administration of dextran sodium sulfate (DSS) do not by any means mimic the etiologies of the two forms of IBD. However, TNBS inflammation mimics several prominent clinical and morphological features of Crohn’s disease (12), whereas DSS inflammation mimics similar features of ulcerative colitis (15, 46). We found that different immune responses mediate the suppression of circular smooth muscle reactivity to ACh in the two models and they target different cell-signaling molecules of the excitation-contraction in circular muscle cells. These findings suggest differential nature of inflammatory mediators that might account for the differences in the two forms of IBD, such as smooth muscle hyperplasia/hypertrophy in Crohn’s

INFLAMMATORY BOWEL DISEASE (IBD), comprised of ulcerative colitis and Crohn’s disease, is chronic, idiopathic, and relapsing inflammation of the gut (34). The two types of IBD are clinically, immunologically, and morphologically distinct. Most Crohn’s colitis patients exhibit transmural colonic inflammation. On the other hand, the general perception is that...
disease and its absence in ulcerative colitis. They also suggest that therapeutic approaches to normalize motility dysfunction may depend on the nature of the inflammatory stimulus that produces it.

MATERIALS AND METHODS

Induction of colonic inflammation by TNBS and DSS in rats. The Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston, TX approved the study using Sprague-Dawley rats (180–280 g, Harlan Sprague Dawley, Indianapolis, IN).

In one group of rats, colonic inflammation was induced by intracolonic administration of TNBS (Sigma Chemical, St. Louis, MO), as described elsewhere (8). We replaced the drinking water with Colyte for 24 h and fasted the animals overnight to cleanse the colon, prior to TNBS (65 mg/kg in 0.25 ml of 40% ethanol) administration under isoflurane anesthesia. We cleansed the colon to minimize its variable absorption by fecal pellets in different rats. We injected TNBS in 1 min through an intracolonic catheter with its tip at 8 cm from the anal margin. The control rats received only 0.25 ml saline. We euthanized the control and inflamed rats at 1, 3, 5, or 7 days after saline or TNBS to obtained tissues for experiments from the distal colon extending 5 cm from the peritoneal reflex.

In the second group of rats, we induced colonic inflammation by oral administration of 5% DSS in drinking water (15, 16). The control rats consumed regular drinking water. We euthanized the control and DSS-treated rats and obtained tissues, as above.

We used a previously described macroscopic scoring system (4) to assess mucosal inflammation as follows: 0 = normal mucosa; 1 = localized hyperemia but no erosions, ulcers, or scars; 2 = linear ulcer or scar with inflammation at one site >2 mm but <5 mm; 3 = two or more sites of ulceration and/or inflammation, each up to 5 mm; 4 = two or more major sites of inflammation and ulcerations >5 mm each or one major site of inflammation extending >1 cm along the length of the mucosa.

Tissue preparations and muscle bath studies. Rat distal colon segments were cleansed and immersed in carbogenated Krebs solution (in mmol/l: 118 NaCl, 4.7 KCl, 2.5 CaC2 1 NaH2PO4, 1.2 MgCl2, 11 D-glucose, and 25 NaHCO3) at room temperature. The mucosa and submucosa were removed under magnifying glass. Circular muscle strips (2 mm × 10 mm) were cut along the circumferential axis and mounted in 5-ml muscle baths (Radnoti Glass, Monrovia, CA) filled with 5 ml carbogenated Krebs solution at 37°C as described previously (42).
Western blotting. The proteins in the samples were resolved by standard immunoblotting method by using 10 or 20 μg in each lane. β-Actin was used as internal control. The antibody (1:200 to 1:400) for α1C subunit of Ca2+,1.2 channels was purchased from Alomone Labs, Jerusalem, Israel (ACC-003). Anti-β-actin and calponin antibodies were purchased from Sigma, anti-CPI-17 and anti-muscarinic M3 receptor antibodies from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-Goq antibody from Calbiochem (Gibbstown, NJ).

Multiplex immunoassay of cytokines and chemokines. Rat colonic muscularis externa or mucosa/submucosa was homogenized in cold PBS supplemented with protease inhibitors for protein extraction. LINCO rat cytokine/chemokine multiplex immunoassay kit (LINCO, St. Charles, MO) was used to quantitate cytokine/chemokine levels in the homogenates by following the manufacturer’s protocols. The assay results were read and analyzed by a Bio-Rad BioPlex System powered by Luminex xMAP Technology (Bio-Rad Laboratories, Hercules, CA).

Immunofluorescence. Tissues were embedded in optimal cutting temperature compound and frozen in liquid nitrogen; 15-μm sections on plus slides were fixed for 20 min in 1 × PBS containing 4% PFA. Primary antibodies were mouse anti-CD163, 1/100 (AbD Serotec, Raleigh, NC) and rabbit anti-elastase, 1/100 (Santa Cruz Biotechnologies); secondary antibodies were donkey anti-mouse Alexa 488 and donkey anti-rabbit Alexa 594 (Invitrogen, Carlsbad, CA). Immunopositive cells in four independently selected high-power fields per animal were counted for three animals per experimental group in the mucosa/submucosa and the muscularis externa.

Measurement of MPO and H2O2. Myeloperoxidase (MPO) level was measured by using the rat MPO ELISA kit from Hycult (Uden, The Netherlands) by following the manufacturer’s instructions. H2O2 concentration was measured by use of the Bioxytech H2O2-560 quantitative hydrogen peroxide assay kit (Oxis International, Foster City, CA). This assay is based on the oxidation of ferrous ions (Fe2+) to ferric ions (Fe3+) by H2O2 under acidic conditions. The ferric ion binds with the indicator dye xylenol orange (3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-α-cresolsulfone-phthalein sodium salt) to form a stable colored complex, which can be measured at 560 nm.

[^3H]Thymidine incorporation assay. Proliferation of rat colonic smooth muscle cells in culture was measured by the incorporation of[^3H]thymidine, as described elsewhere (25). Briefly, the cells were incubated for 24 h in serum-free DMEM. Quiescent muscle cells were incubated with IGF-I (10–100 ng/ml), TGF-β (1–10 ng/ml), TNF-α (20 ng/ml), or IL-1β (10 ng/ml) for 24 h. During the final 8 h, 1 μCi/ml[^3H]thymidine was added to the medium.[^3H]Thymidine incorporation into the perchloric acid extractable pool was used as a measure of new DNA synthesis.

Statistical analysis. All data are expressed as means ± SE. We used analysis of variance with nonrepeated measures followed by Student-Newman-Keuls test for multiple comparisons and Student’s test for comparison of two means. P value of < 0.05 was considered statistically significant.

RESULTS

Inflammatory responses in TNBS- and DSS-induced colonic inflammations. The thickness of the muscularis externa increased significantly on day 7 of TNBS inflammation (89 ± 3 to 166 ± 10 μm, n = 6 P < 0.05), but that in DSS inflammation was not different from the control (89 ± 3 to 98 ± 4 μm, n = 6, P > 0.05) (Fig. 1, A–C). Hematoxylin and eosin staining showed extensive infiltration of inflammatory cells in the muscularis externa on day 7 of TNBS inflammation, compared with that in saline-treated rats (Fig. 1, D and E). By contrast, the density of inflammatory cells in the muscularis externa of DSS inflammation did not appear to be different from that in saline-treated controls (Fig. 1, D and F). However, the mucosal injury scores based on macroscopic observations on day 7 were not significantly different between the two models (4.6 ± 0.4 in TNBS rats and 4.2 ± 0.3 in DSS rats, P < 0.05 vs. 0. ± 0.9 in controls, n = 6 in each group). The growth in body weight was also not significantly different in the two groups of rats (112 ± 6% in TNBS rats and 116 ± 5% in DSS rats).

**Fig. 2. Effects of 68 mg/kg TNBS and 5% DSS on the contractile response of colonic circular smooth muscle strips to acetylcholine (ACH) on days 1, 3, 5, and 7 of inflammation (Inf.). AUC, area under contractions normalized by dry tissue weight. N = 4 or 5 rats at each time point. *P < 0.05 vs. saline-treated controls.**
Differential mechanisms of smooth muscle dysfunction in TNBS and DSS colonic inflammations. Despite differences in the intensities of infiltration of the immune cells and in the expression and time course of MPO in the muscularis externa and mucosa/submucosa in the two models, the circular smooth muscle contractility was impaired in both groups of rats. The reactivity to ACh decreased on day 1 in TNBS rats, which sustained for 7 days (Fig. 2, A–D). On the other hand, the reactivity to ACh decreased progressively in DSS rats from day 1 to day 7 (Fig. 2, E–H). However, on day 7 of inflammation, the suppression of reactivity to ACh was of the same order of magnitude in both types of inflammation (24 ± 4 and 33 ± 6% at 10⁻² M ACh of control).

Differential expressions of inflammatory mediators in the two models of inflammation. Oxidative stress and peptide mediators are two prominent processes that induce inflammation. We investigated whether these two classes of inflammatory mediators play differential roles in suppressing the reactivity of smooth muscle cells to ACh in TNBS and DSS inflammations. We found that the concentrations of several prominent cytokines and chemokines [IL-1β, TNF-α, IL-6, monocyte chemotactrant protein (MCP-1), and IL-8] increased significantly in the mucosa/submucosa and muscularis externa from day 1 to day 7 of TNBS inflammation (Fig. 3, A–D). On the other hand, the concentration of TNF-α increased significantly on days 1 and 3 only in the mucosa/submucosa (Fig. 3E). IL-4 concentration did not change in any tissue in TNBS inflammation (Fig. 3F).

By contrast, DSS inflammation induced markedly smaller increases of IL-1β, MCP-1, and IL-8 (Fig. 3, A, C, and D) and no increase in IL-6 in the mucosa/submucosa (Fig. 3B). However, the concentrations of TNF-α and IL-4 increased signifi-

![Graphs and images showing cytokine expression](http://ajpgi.physiology.org/)
cantly in this tissue on day 7 of inflammation (Fig. 3, E and F). By contrast, none of these cytokine inflammatory mediators showed a significant increase in the muscularis externa of the DSS rats (Fig. 3, A–F).

The oxidative stress measured as the concentration of H2O2 increased significantly in the mucosa/submucosa and the muscularis externa throughout the 7-day period of observation in TNBS inflammation (Fig. 4, A and B). The mucosa/submucosa of DSS inflammation showed a similar pattern of increase in H2O2 (Fig. 4C). However, the concentration of H2O2 in the muscularis externa of DSS inflammation exhibited a gradual increase from day 1 to day 7 (Fig. 4D). The maximum increase of H2O2 in both types of inflammation and tissue layers was about the same order of magnitude.

Immunostaining with CD163 for activated macrophages on full thickness sections showed a small but significant increase of activated macrophages in the mucosa/submucosa on day 3, which increased severalfold on day 7 of TNBS inflammation (Fig. 5, top graph). Small but significant increases of activated macrophages in DSS inflammation occurred on days 1 and 3 in this type of inflammation. On day 7, the increase of macrophages in TNBS inflammation was severalfold greater than that in DSS inflammation. The macrophages showed a small but significant increase in the muscularis externa of TNBS on day 3 of inflammation, which increased severalfold on day 7 (Fig. 5, bottom graph). By contrast, only a small but significant increase in macrophages occurred in the muscularis externa on day 7 of DSS inflammation (Fig. 5, bottom graph).

Immunostaining with elastase showed that the significant increase of neutrophils in mucosa/submucosa of TNBS rats peaked on day 1, which declined steadily by day 7 of inflammation (Fig. 6, top graph). DSS rats exhibited a minor, but significant, increase of neutrophils in mucosa/submucosa only on day 3 of inflammation (Fig. 6, top graph). TNBS inflammation showed a minor increase of neutrophils in the muscularis externa on day 3 (Fig. 6, bottom graph). By contrast, neutrophils did not increase in the muscularis externa of DSS inflammation during the 7 days of observation (Fig. 6, bottom graph).

Molecular targets for the suppression of reactivity to ACh in TNBS- and DSS-induced inflammation. The cell signaling pathways of the excitation-contraction coupling that converge on the phosphorylation/dephosphorylation of the 20-kDa regulatory light chain (RLC20) regulate the amplitude of smooth muscle contraction in response to ACh. We investigated whether the inflammatory mediators in the two models of inflammation differentially alter the expression of four key cell-signaling proteins of the excitation-contraction coupling [muscarinic M3 receptor, Gαq, α1C subunit of Ca1.2b (L-type) channels, and CPI-17] to suppress smooth muscle reactivity to ACh. We found that TNBS inflammation significantly suppresses expression of the α1C subunit, Gαq, and CPI-17 (Fig. 7A). However, it has no significant effect on the expression of muscarinic M3 receptor. By contrast, DSS inflammation significantly suppresses only the expression of Gαq (Fig. 7B).

Next, we investigated whether IL-1β and H2O2 suppress the reactivity of circular smooth muscle to ACh in vitro by differentially altering the expression of cell signaling proteins of the excitation-contraction coupling. The incubation of naive circular muscle strips from the distal rat colon with 20 ng/ml IL-1β or 200 μM H2O2 for 24 h significantly suppressed their reactivity to ACh (Fig. 8). The incubation with IL-1β suppressed the expression of α1C subunit, Gαq (Fig. 8), and CPI-17 (data not shown), whereas the incubation with H2O2 suppressed the expression only of Gαq (Fig. 8). The expression of muscarinic M3 receptor did not change in response to IL-1β or H2O2.

Differential smooth muscle hyperplasia and hypertrophy in the two models of inflammation. We investigated whether differential expressions of growth factors IGF-1 and TGF-β account for the induction of hyperplasia and hypertrophy in TNBS inflammation, but not in DSS inflammation. Both IGF-1 and TGF-β were elevated throughout the 7 days of TNBS inflammation, peaking on day 1 (Fig. 9A). They showed no

---

Fig. 4. Quantitative data of H2O2 (μM/mg of protein) in the colonic mucosa/submucosa and muscularis externa in TNBS and DSS inflammation over 7 days. *P < 0.05 vs. saline-treated controls.
significant change in DSS inflammation (Fig. 9B). In vitro incubation of smooth muscle cell cultures with IGF-1 concentration dependently enhanced the proliferation of smooth muscle cells, whereas incubation with TGF-β had no significant effect (Fig. 9, C and D). The incubations of cell cultures with 1–50 ng/ml IL-1β or TNF-α for the same duration had no significant effect on cell proliferation (data not shown). The incubation of cell cultures with IGF-1 or TGF-β enhanced the expression of calponin, a marker of hypertrophy (29) (Fig. 9, E and F).

DISCUSSION

TNBS and DSS induced inflammations mimic Crohn’s disease-like and ulcerative colitis-like morphological and functional features (12, 15, 46). These models have been instrumental in advancing our understanding of the nature of inflammatory responses in the two types of IBD and in developing therapeutic agents to treat them. There is little doubt that both types of gut inflammation begin in the mucosa, which separates the normally sterile gut wall structures from the luminal pathogens. The conventional concept is that the inflammation in Crohn’s disease is transmural, whereas that in ulcerative colitis is limited largely to mucosa. This concept might have developed from morphological observations of significant infiltration of white blood cells in the muscle layers of Crohn’s disease, but not in those of ulcerative colitis. However, this concept is not consistent with the fact that circular smooth muscle contractility is suppressed in both types of inflammation (1, 44, 48, 50).

Our findings suggest that both models portray transmural inflammation, if inflammation is defined as that due to the infiltration of white blood cells or the presence of oxidative stress. H2O2 concentration peaked early on in the mucosa/submucosa in DSS inflammation, and from there it propagated and gradually built up in the muscularis externa in 5 days. The increase of oxidative stress in the muscularis externa occurred in the absence of any activated immune cells. Except for a late increase of TNF-α, the concentrations of cytokines/chemokines in the mucosa/submucosa in this type of inflammation were severalfold smaller than those in Crohn’s disease-like inflammation. The concentrations of none of the cytokines/chemokines that we examined increased in the muscularis externa of the ulcerative colitis-like inflammation. By contrast, both oxidative stress and concentrations of cytokines increased

Fig. 5. CD163 (green) immunofluorescence in distal colon sections from control, TNBS-treated, and DSS-treated rats 1, 3, and 7 days after inflammatory insult. Graphs display the average numbers of CD163-immunoreactive cells (macrophages) per high-powered field (HPF) in either the mucosa/submucosa or the muscularis externa observed in 3 animals per time point, 3 slides per animal. *P < 0.05 vs. control; #P < 0.05 TNBS vs. DSS. CM, circular muscle; LM, longitudinal muscle.
at similar intensities in the mucosa/submucosa and muscularis externa of Crohn’s disease-like inflammation.

In contrast to the propagation of oxidative stress from the mucosa/submucosa to the muscularis externa over a period of 5 days in DSS inflammation, the oxidative stress and cytokine/chemokine concentrations increased almost concurrently in the mucosa/submucosa and muscularis externa of TNBS inflammation. Garcia-Lafuente et al. (14) reported that luminal bacteria translocate across the colon wall within 24 h in TNBS inflammation. They also found that TNBS impairs epithelial barrier function by necrosis (14). By contrast, Toll-like receptor 4 (TLR4) signaling, which limits bacterial translocation, mediates DSS inflammatory response (13, 37). DSS arrests the epithelial cell cycle, resulting in apoptosis, impaired proliferation, and weak release of inflammatory mediators (2, 23, 49). DSS inflammation occurs in germ-free or severely combined immunodeficiency (scid) mice (11, 22). Consequently, bacterial translocation is marginal and confined to the mucosa so that it plays a lesser role in DSS inflammation than in TNBS inflammation. Taken together, aggressive bacterial translocation in TNBS inflammation may underlie the transmural infiltration of immune cells and release of cytokines/chemokines. On the other hand, limited bacterial translocation results in much smaller infiltration of immune cells and release of cytokines/chemokines in the mucosa/submucosa of DSS inflammation. It is noteworthy that TNBS inflammation in the absence of intestinal flora is also primarily mucosal (14). Our data suggest that the cytokines/chemokines in the mucosa/submucosa do not diffuse to the muscularis externa. By contrast, H₂O₂ diffuses through membranes and extracellular space because of its high biomembrane permeability (7). The differences in the nature of damage to the epithelium, such as apoptosis and necrosis, may underlie the two strikingly different types of responses in TNBS and DSS inflammations.

The profiles of activated immune cells and oxidative stress in our models might relate to the initial episode of inflammation in IBD patients. Accumulating evidence suggests that each inflammatory episode modifies the subsequent inflammatory response in IBD (3). Therefore, the profiles of immune cells and oxidative stress are likely to differ at different stages of the human disease. Human biopsies for investigation are available usually after repeated episodes of inflammation. However, it seems that the basic nature of inflammatory responses in the two types of inflammation persists after repeated relapses because the muscularis externa in established ulcerative colitis...
Fig. 7. Effects of TNBS (A) and DSS (B) inflammation on the expression of 4 proteins (α1C, CPI-17, Gαq, and muscarinic receptor M3) of the excitation-contraction coupling over 7 days. The muscularis externa tissue was collected from rat distal colon, and the protein extracts were used for the Western blotting. N = 4 or 5 rats for each time point. *P < 0.05 vs. saline controls.

Fig. 8. In vitro effect of exogenous IL-1β (A) and H2O2 (A) on smooth muscle contractility and expression of α1C and Gαq in (B) rat colonic circular muscle strips. Naive colonic circular muscle strips were incubated in DMEM in the absence and presence of IL-1β (10 ng/ml) and H2O2 (100 μM) at 37°C. The circular smooth muscle reactivity to ACh and expression of α1C and Gαq were determined 24 h later. N = 3 independent experiments. *P < 0.05 vs. medium controls.
patients does not develop hypertrophy/hyperplasia, whereas the reactivity to ACh remains suppressed in established ulcerative colitis and Crohn’s disease patients (1, 44, 48, 50). Regardless, qualitative snapshots of distribution of immune cells at various stages of Crohn’s disease or ulcerative colitis (5) might be of little help in uncovering their etiologies or suggesting target molecules for therapy.

We found lack of correlation between the intensity of immune cell infiltration and increase in concentration of inflammatory mediators. The inflammatory mediators peaked earlier in inflammation than the infiltration and activation of immune cells. These findings suggest that the inflammatory mediators released from the initial infiltration of immune cells might recruit nonimmune cells to rapidly enhance their concentrations and mature the inflammatory response. Numerous studies have established that the nonimmune cells, such as smooth muscle, cardiac muscle, myofibroblast, and epithelial cell, secrete significant amounts of pro- and anti-inflammatory cytokines (9, 35, 39, 43, 47). Taking into account the much larger combined volume of nonimmune cells in the gut wall compared with that of the resident and infiltrating immune cells, the secretions of inflammatory mediators by the nonimmune cells may play a prominent role in the intensity and time course of the overall inflammatory response. In this context, the nonimmune cells may serve as effective targets of therapeutic agents to suppressing inflammation.

Smooth muscle function is impaired in both Crohn’s disease and ulcerative colitis patients (1, 38, 44, 48, 50) as well as in TNBS and DSS models (16, 20, 21, 24, 27), which supports our hypothesis that inflammation, defined as that due to infiltrating white blood cells or generation of oxidative stress is transmural in both types of inflammations. Even though the smooth muscle contractility is impaired in both models of inflammation, the cellular and genetic mechanisms differ. In TNBS inflammation, the cytokines, such as IL-1β, impair smooth muscle function by suppressing expression of the α1c1b subunit of Ca_v1.2b (L-type) calcium channels, CPI-17 and Go_q. By contrast, oxidative stress in DSS inflammation impairs smooth muscle function by suppressing expression of the Go_q protein. Note that TNBS inflammation suppresses Go_q because it also generates oxidative stress of the same order as DSS inflammation.

Our findings show that smooth muscle dysfunction in DSS inflammation results primarily from oxidative stress. 1) No significant increase of proinflammatory cytokines occurred in this tissue, and 2) the suppression of contractility increased gradually from day 1 to day 7, which is similar to the increase of H_2O_2 in this tissue. By contrast, impairment of smooth...
muscle function in TNBS inflammation results from both oxidative stress and proinflammatory cytokines. This type of inflammation suppresses both α1C-subunit and Gαq, which are regulated by IL-1β and H2O2, respectively.

Oxidative stress plays a key role in a number of diseases, including diabetes (41), peritoneal endometriosis (31), and atherosclerosis (18). However, we do not know the underlying mechanisms of generation of primarily oxidative stress by DSS and of identical oxidative stress accompanied with massive recruitment and activation of immune cells by TNBS. These mechanisms might underlie the different etiologies of ulcerative colitis and Crohn’s disease.

The hyperplasia/hypertrophy of smooth muscle cells in Crohn’s disease and its absence in ulcerative colitis is a classic marker of the differences between the two forms of IBD (26, 28). The TNBS and DSS models, respectively, replicate these features. Our findings show that these differences relate to differential generation of growth factors in the two models; TNBS inflammation enhances the expressions of growth factors, such as IGF-1 and TGF-β in the muscularis externa. Our in vitro studies confirm that IGF-1 induces the proliferation of smooth muscle cells in culture (19), whereas TGF-β has no effect. On the other hand, both IGF-1 and TGF-β induce hypertrophy. We do not know the precise mechanisms of generation or the source of growth factors. However, we found that the cytokines IL-1β and TNF-α do not induce proliferation in smooth muscle cells. By contrast, oxidative stress in DSS inflammation does not generate growth factors in the muscularis externa, and, therefore, it does not exhibit thickening of the muscularis externa.

On the basis of extensive scientific and clinical evidence accumulated over decades, Pravda (36) makes a compelling case that increase in the production of oxidative stress in epithelial cells followed by diffusion to its microenvironment, damage to tight junctions and local accumulation of white blood cells underlie the etiology of ulcerative colitis (radical induction theory of ulcerative colitis). Our findings support and extend this theory by showing that excess H2O2 generated initially in the epithelial cells recruits other nonimmune cells (smooth muscle, enteric neurons, myofibroblasts, and glia) and a small number of infiltrating immune cells to enhance its generation and transmural diffusion, resulting in neuromuscular dysfunction. Even though some neutrophils and macrophages infiltrate the mucosa/submucosa, they may not underlie colonic motility dysfunction in ulcerative colitis. The current paradigm is that imbalances in Th1-Th2 responses distinguish the two types of IBD: ulcerative colitis and Crohn’s disease. Accumulative evidence suggests a shift in this paradigm. An imbalance in the generation and neutralization of free radicals may underlie ulcerative colitis, whereas Th1 response may underlie Crohn’s disease. Some reports have challenged the Th1-Th2 paradigm because of inconsistencies (3, 17). The relative efficacies of current therapies in the two forms of IBD support the suggested shift in paradigm. 5-Aminosalicylic acid (5-ASA) compounds are effective in ulcerative colitis, whereas their efficacy in Crohn’s disease remains controversial (5). 5-ASA compounds show reactive oxygen species’ scavenging capabilities among their numerous modes of action (30). The relative role of oxidative stress in Crohn’s disease is not established.

The classic definition of inflammation is based on the recruitment or activation of immunocytes. The activated immune cells release cytokines, which have protective and deleterious effects on the surrounding tissues and define the time course of the inflammatory response/disease. However, it is now clear that the immune cells recruit surrounding nonimmune cells to mature the inflammatory response. The immune cells also generate reactive oxygen species, which have protective and deleterious effects on the surrounding tissues, similar to those of peptide inflammatory mediators (45). In addition, reactive oxygen species form as natural by-products of cellular metabolism of oxygen, which are predominantly neutralized by the cellular antioxidant defense system. However, an imbalance in this system results in excessive oxidative stress independent of activation of immune cells. This oxidative stress defines the course of the inflammatory response in much the same way as that initiated by immune cells. Using the extended definition of inflammation, our findings and available clinical data (6) show that inflammation is transmural in ulcerative colitis and its model of DSS inflammation.

In conclusion, our findings show that both TNBS and DSS inflammations induce transmural inflammation. However, the inflammatory response in DSS inflammation is primarily due to the generation of transmural oxidative stress. On the other hand, in TNBS inflammation it is due to the generation of transmural oxidative stress and release of proinflammatory cytokines/chemokines. The increases in the concentrations of peptide inflammatory mediators in the colon wall do not correlate with the increase in the infiltration or activation of resident and infiltrating macrophages and neutrophils. It seems that the initial release of inflammatory mediators from immune cells may recruit nonimmune cells to secrete proinflammatory mediators. The oxidative stress and proinflammatory cytokines similarly impair smooth muscle function, which contributes to diarrhea. However, they target different proteins of the excitation-contraction coupling to suppress smooth muscle reactivity to ACh. The hyperplasia in TNBS inflammation is due to the generation of IGF-1 in muscularis externa. Both IGF-1 and TGF-β induce hypertrophy.

GRANTS

This research was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK 32346.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

6. Cao W, Fiocchi C, Pricolo VE. Production of IL-1β, hydrogen peroxide, and nitric oxide by colonic mucosa decreases sigmoid smooth muscle.