The Vagus Nerve: A Tonic Inhibitory Influence Associated With Inflammatory Bowel Disease in a Murine Model

JEAN ERIC GHIA, PATRICIA BLENNERHASSETT, HARRY KUMAR-ONDIVEERAN, ELENA F. VERDU, and STEPHEN M. COLLINS
Intestinal Diseases Research Programme, Health Science Center, McMaster University, Hamilton, Ontario, Canada

Background & Aims: The recently proposed Inflammatory Reflex describes an interaction between the vagus nerve and peripheral macrophages, resulting in attenuation of proinflammatory cytokine release in response to systemic exposure to bacterial endotoxin. The purpose of this study was to determine whether a similar vagus/macrophage axis modulates the inflammatory responses in the colon in mice. Methods: We assessed the Disease Activity Index (DAI), macroscopic and histologic damage, serum amyloid-P level, and myeloperoxidase activity in colitis induced by administration of dextran sodium sulfate (DSS) in healthy and vagotomized C57BL/6 and in mice deficient in macrophage-colony stimulating factor (M-CSF)-induced and in hapten-induced colitis. A pyloroplasty was performed in vagotomized mice. Results: DAI, macroscopic and histologic scores, myeloperoxidase activity, levels of serum amyloid-P, and colonic tissue levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α were increased significantly in vagotomized mice 5 days post-DSS and 3 days after hapten-induced colitis compared with sham-operated mice that received DSS or the hapten. Pretreatment with nicotine significantly decreased each of these markers in vagotomized mice with DSS colitis, and all markers except DAI and IL-6 in sham-operated DSS-treated mice. Conversely, hexamethonium treatment significantly increased each of these markers in the sham-operated DSS-treated mice. Vagotomy had no effect on the colitis in M-CSF–deficient mice. Conclusions: The vagus nerve plays a counterinflammatory role in acute colitis via a macrophage-dependent mechanism, involving hexamethonium-sensitive nicotinic receptors. The identification of a counterinflammatory neural pathway would open new therapeutic avenues for treating acute exacerbations of inflammatory bowel disease.

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory disorder that affects up to 500 per 100,000 persons in Western countries, and is considered the consequence of an aberrant immune response to luminal antigens. Tissue injury results from the release of inflammatory mediators, including acid metabolites and proinflammatory cytokines. In particular, increased secretion of proinflammatory cytokines is considered to be important for exacerbation of IBD, and several assays of therapeutic approaches targeting inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α), have been investigated extensively. Cytokine production can be modulated by neurotransmitters, including those of the autonomic nervous system. The autonomic nervous system is altered both structurally and functionally in IBD; structural changes in autonomic nerves in the gut include changes in ganglia size and number as well as axonal necrosis. Up to 35% of patients with ulcerative colitis (UC) show autonomic with impaired parasympathetic function, resulting in sympathetic dominance. Studies in animal models have raised the possibility of autonomic imbalance, which contributes to the inflammatory drive in IBD. This is based on observations that the sympathectomy improves experimental colitis and that administration of the parasympathomimetic nicotine improves colitis in the animal model.

Recently, attention has focused on the role of the vagus nerve in modulating inflammatory responses. Based on studies of cytokine release after systemic exposure to bacterial lipopolysaccharide, Tracey postulated the existence of a vagally mediated anti-inflammatory reflex in which the presence of proinflammatory cytokines in the periphery is detected by vagal afferents, resulting in a vagal efferent response associated with an attenuation of cytokine release from macrophages via nicotinic acetylcholine receptors. It has been shown that vagotomy (VX) accelerated lipopolysaccharide-induced septic shock and increased systemic TNF-α production, and that selective inhibition of vagal afferent fibers worsened hapten-induced colitis. Conversely, the stimulation of the vagus nerve selectively down-regulated production of proinflammatory cytokines.

This vagal reflex involves the release of acetylcholine, which interacts with the α 7 subunit nicotinic receptor on macrophages. This is supported further by demonstrations in vitro that nicotinic receptors are involved in the selective down-regulation of lipopolysaccharide-induced release of TNF-α, IL-6, and IL-1β in cultured macrophages.

Macrophages are an important component of the inflammatory response in IBD. Recruited macrophages are evident in the lamina propria in tissues from patients with IBD, and those cells are responsible for the production of proinflammatory cytokines.

The existence of the vagal inflammatory reflex has been promoted in the context of the acute paradigm of septic shock. The extent to which a similar vagus–macrophage axis exists in the context of intestinal inflammation remains to be determined. Thus, the present study examined the role of the vagus in 2 models of colitis induced by a lymphocyte-independent

Abbreviations used in this paper: CCK, cholecystokinin octapeptide; DAI, Disease Activity Index; DNBS, dinitrobenzene sulfonic acid; DSS, dextran sodium sulfate; IBV, irritable bowel disease; IL, interleukin; M-CSF, macrophage colony stimulating factor; MPO, myeloperoxidase; SAP, serum amyloid-P; TNF, tumor necrosis factor; VX, vagotomy; VXP, vagotomiy and pyloroplasty.
model by oral administration of dextran sodium sulfate (DSS) and by a lymphocyte-dependent model using intra-colonic administration of dinitrobenzene sulfonic acid (DNBS), and determined whether a macrophage subset was involved.

### Materials and Methods

#### Animals

Male C57BL/6 mice (7–9 weeks old) were purchased from Harlan Animal Suppliers (Indianapolis, IN) and maintained in the animal care facility at McMaster University under specific pathogen-free conditions. Macrophage-Colony Stimulating Factor (M-CSF)-C57BL/6 deficient (op/op) and heterozygote op/+ breeding pairs were purchased from Jackson (Bar Harbor, ME). Nonmutant mice (op/+ or +/+ ) are indistinguishable from each other and therefore are named op/-. Op/op mice have osteopetrosis and lack teeth; they were therefore fed a powdered diet, whereas op/- mice received conventional food. No differences in food intake or body weight were observed between these groups. Mice were housed under standard conditions for a minimum of 1 week before experimentation. All experiments were approved by the McMaster University Animal Research Ethics Board and were conducted under the Canadian Council on Animal Care Guidelines.

#### Vagotomy

Mice were anesthetized using ketamine (150 mg/kg, intraperitoneally [IP]) and xylazine (10 mg/kg, IP) and ventral and dorsal truncal branches of the subdiaphragmatic vagus were cut (1 cm above the gastroesophageal junction). Preliminary studies showed marked gastric dilatation in vagotomized mice and a surgical pyloroplasty was therefore incorporated into the protocol. VX and pyloroplasty (VXP) subsequently were performed under the same anesthesia. No gastric dilatation was observed in mice undergoing this procedure. In sham-operated mice, vagal trunks were similarly exposed but not cut, but a pyloroplasty was performed. All mice were maintained on normal diet.

#### Validation of VX

The ability of cholecystokinin octapeptide (CCK-8) to reduce food intake is completely dependent on the integrity of the vagus nerve. To determine the functional integrity of VX in our study, mice received 40 µg/kg IV of CCK-8 (Sigma, Oakville, Ontario, Canada) 10 days after VX or sham surgery and food intake was measured over 24 hours. The integrity of VX lasts well beyond the time frame of the present studies, and for as long as 62 days (Ghia et al, unpublished observation). The functional integrity of VXP was ascertained by the absence of a CCK-8-induced suppression of feeding. The completeness of VX was verified during postmortem inspection of vagal nerve endings using microscopic inspection.

#### Induction of DSS and DNBS Colitis

Two days after the end of the CCK-8 experiment, DSS (molecular weight, 40 kilodaltons; ICN Biomedicals Inc, Aurora, OH) was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days. Controls were all time-matched and consisted of mice that received normal drinking water only. Mean DSS consumption was noted per cage each day. For the DNBS study, mice were anesthetized with enflurane (Abbott, Abbott Park, IL). A 10-cm long PE-90 tubing (ClayAdams, Parsippany, NJ), attached to a tuberculin syringe, was inserted 3.5 cm into the colon. Colitis was induced by administration of 100 µL of 4 mg of DNBS solution (ICN, Biomedical Inc) in 30% ethanol for 3 days. Control mice (without colitis) received saline. Mice with colitis were supplied with 8% sucrose in drinking water to prevent dehydration.

#### Administration of Parasympathetic Drugs

Exposure to DSS (%) commenced on the 14th day after surgery and continued for 5 days. In separate experiments, nicotine (20 µg/mL) was added to the drinking water 10 days before and for 5 days after the induction of colitis in VXP. In separate experiments, hexamethonium (10 mg/kg) was administered by subcutaneous (SC) injection twice a day for 5 days post-DSS in sham-operated mice.

#### Assessment of the Severity of Colitis: Disease Activity Index

Disease Activity Index (DAI) scores historically have correlated well with the pathologic findings in a DSS-induced model of IBD. DAI is the combined score of weight loss, stool consistency, and bleeding. Scores were defined as follows: for weight: 0, no loss; 1, 5%–10%; 2, 10%–15%; 3, 15%–20%; and 4, 20% weight loss; for stool: 0, normal; 2, loose stool; and 4, diarrhea; and for bleeding: 0, no blood; 1, 20% weight loss; for stool: 0, normal; 2, loose stool; and 4, diarrhea; and for bleeding: 0, no blood; 2, presence (Hemoccult II positive; Beckman Coulter, Fullerton, CA); and 4, gross blood. DAI was scored from days 0–5 during DSS treatment.

#### Serum Amyloid-P Assay

Blood was collected either 5 or 3 days after the beginning of the DSS or DNBS treatment, respectively, by intracardiac puncture in anesthetized (enflurane) mice. Serum amyloid-P (SAP), a major acute-phase protein in mice, was measured by an enzyme-linked immunosorbent assay as an acute inflammatory marker. A 96-well microplate (MaxiSorp; Nunc, Naperville, IL) was coated with a sheep anti-mouse SAP polyclonal antibody (Calbiochem, San Diego, CA) overnight. One-hundred microliters of either SAP standard or serum samples from mice were added and then incubated for 1.5 hours at room temperature. A rabbit anti-mouse SAP polyclonal antibody (Calbiochem) was added, and the plate was incubated for another 1.5 hours at room temperature. A horseradish-peroxidase–conjugated mouse anti-rabbit IgG (chain specific) monoclonal antibody (Sigma) was added, and the plate was incubated for another 1.5 hours at room temperature. Finally, substrate solution containing o-phenylenediamine (Sigma) was added to each well and incubated for 30 minutes at room temperature. The absorbance at 492 nm was measured with a microplate reader. Standard SAP samples were used to create a standard curve. The colon was removed and divided and used for the following measurements.

#### Macroscopic Scores

Five days after the beginning of the DSS or 3 days after the beginning of the DNBS treatment, the mice were killed and the abdominal cavity was opened, the colon was located, and observations on distension, fluid content, hyperemia, and erythema were recorded. The colon was removed and opened longitudinally, and macroscopic damage was assessed immedi-
Colonic Histology and Myeloperoxidase Activity

Formalin-fixed colon segments were paraffin-embedded and 3-µm sections were stained with H&E. Colonic damage was scored based on a published scoring system that considers architectural derangements, goblet cell depletion, edema/ulceration, and degree of inflammatory cell infiltrate. Myeloperoxidase (MPO) activity was determined following an established protocol. Briefly, MPO activity, used as a marker of neutrophilic infiltration, was extracted and the activity was measured using a modified version of the method described by Bradley et al. Tissue samples were homogenized (50 mg/mL) in ice-cold 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide (Sigma). The homogenate was freeze-thawed 3 times, briefly sonicated, and then centrifuged at 12,000 rpm for 12 minutes at 4°C. The supernatant then was added to a solution of O-dianisidine (Sigma) and hydrogen peroxide. The absorbance of the colorimetric reaction was measured by a spectrophotometer. MPO is expressed in units per milligram of wet tissue, 1 unit being the amount of enzyme able to convert 1 µmol of hydrogen peroxide to water in 1 minute at room temperature.

Cytokine Levels

The colonic sample was homogenized in 700 µL of Tris-HCl buffer containing protease inhibitors (Sigma). Samples were centrifuged for 30 minutes, and the supernatant was frozen at 80°C until assay. Cytokine levels (IL-1β, IL-6, and TNF-α) were determined using an enzyme-linked immunosorbent assay commercial kit (Quantikine M murine; R&D Systems, Minneapolis, MN).

Statistical Analysis

Results are presented as means ± SD. Statistical analysis was performed using 1-way analysis of variance followed by the Student–Newman–Keuls multiple comparisons post hoc analysis and a P value of less than .05 was considered significant.

Results

Responses to CCK-8

Food intake was decreased significantly by 84.2% ± 1.8% after CCK-8 injection in sham-operated mice compared with VXP mice (+2.7% ± 1.1% (data not shown). The VXP mice in which CCK induced a significant reduction in food intake were excluded from subsequent studies on the assumption that the vagotomy was incomplete. In contrast, water intake was not different between VXP and sham-operated mice (5.3 ± 0.6 and 6.5 ± 0.3 mL/24 h, respectively).

Effect of VXP Without Colitis

VXP caused no changes in weight gain, colonic appearance, histology, SAP, MPO, or cytokine levels in C57BL/6 mice without colitis. TNF-α, IL-1β, and IL-6 colonic tissue levels were below the lowest standard of the assay in these mice (data not shown).

The Effect of VXP on DSS-Induced Colitis

DSS induced a colitis characterized by weight loss and frequent stools; this was evident by day 3 in sham-operated mice. In VXP mice, the onset of colitis was accelerated, as injury reflected in the DAI were seen within 2 days of DSS. As shown in Figure 1A, the DAI was significantly higher in VXP mice compared with the sham-operated mice on each of the 5 days of colitis, the differences between groups reached statistical significance on all 5 days of DSS regimen. As shown in Figure 1B, VXP increased the macroscopic scores significantly at day 5 after DSS. DSS increased MPO activity from 0.25 ± 0.15 U/mg in control mice to 2.57 ± 0.51 U/mg. As shown in Figure 1C, VXP resulted in significantly higher MPO activity of 7.22 ± 1.73 U/mg compared with sham-operated mice. SAP levels increased from 31.5 ± 8.9 in DSS sham-operated mice to 154.9 ± 19.4 µg/mL in DSS-VXP mice (Figure 1D). As shown in Figure 2, VXP significantly increased the severity of colitis with histologic scores increasing from 1.6 ± 0.6 to 2.7 ± 0.5 (Figure 2A). This was associated with a greater loss of tissue architecture, edema, and a massive, mixed immune cell infiltrate (mononuclear cells, neutrophils, and eosinophils) (Figure 2B). In addition, we found significantly greater fold increases in the levels of TNF-α (4.5-fold), IL-1β (3.2-fold), and IL-6 (11.4-fold) in the colon of DSS-treated mice with VXP compared with sham-operated mice (Figure 3).

Effects of Nicotine and Hexamethonium

Nicotine treatment (20 µg/mL) significantly decreased the DAI during the first 3 days. Nicotine also significantly decreased the macroscopic damage scores in VXP-DSS-treated mice compared with VXP mice with DSS colitis; only macroscopic changes were reduced significantly in sham-operated DSS-treated mice (Figures 1A and B). MPO activity was decreased significantly in both VXP and sham-operated mice treated with nicotine during DSS treatment (3.77 ± 0.16 U/mg) (Figure 1C). Nicotine also reduced SAP levels significantly in VXP and sham-operated DSS-treated mice (Figure 1D). These mice also showed an improvement in histologic scores (Figure 2A). Nicotine treatment reduced the levels of all 3 proinflammatory cytokines in VXP mice significantly, but only TNF-α and IL-1β in sham-operated DSS-treated mice (Figure 3). Hexamethonium (10 mg/kg) was administered to sham-operated DSS mice and significantly increased the macroscopic score (Figure 1B), MPO activity (Figure 1C), and SAP levels (Figure 1D) compared with saline-treated mice. Hexamethonium also significantly increased histologic damage scores (Figure 2A) and the levels of IL-1β, TNF-α, and IL-6 as shown in Figure 3.

Role of Macrophage Effect of DSS in M-CSF-Deficient Mice

To elucidate the role of the macrophage we used mice deficient in M-CSF. DSS induced significant colitis in both op/op and op/? mice, although more inflammation was seen in the op/? mice. As shown in Figures 4A and B, DSS 5% significantly increased macroscopic and histologic damage scores at day 5 in op/op and op/? mice. The histologic score increased from 0.45 ± 0.33 to 1.24 ± 0.15 for the op/op group and from 0.3 ± 0.3 to 2.12 ± 0.22 in op/? mice. Similarly, DSS 5% significantly increased MPO activity (Figure 4C) and SAP levels (Figure 4D).
Effect of VXP on DSS-Induced Colitis on M-CSF-Deficient Mice

Op/op mice developed a slower onset of DSS-induced colitis compared with op/? mice, as shown in Figure 5A. In contrast to the increased severity of colitis seen in op/? mice with VXP compared with sham-operated op/? mice, VXP had no effect on the severity of colitis in op/op mice. Similarly, there was less macroscopic damage in op/op mice with colitis compared with op/? (Figure 5B). In keeping with this pattern, MPO activity in op/op mice was lower compared with op/? (1.23 ± 0.31 and 2.04 ± 0.54 U/mg, respectively). As shown in Figure 5C, VXP increased MPO in op/? mice with colitis but it had no effect in op/op mice. Measurements of SAP and cytokine levels followed a similar pattern, as shown in Figure 5D and Figure 3. Histologic damage scores in op/op DSS-treated mice were decreased significantly compared with op/? from 2.12 ± 0.22 to 1.24 ± 0.15 and VXP in op/op DSS-treated mice did not alter this difference, as shown in Figure 2B.

Effect of VXP on DNBS-Induced Colitis

As shown in Table 1, VXP significantly increased the macroscopic scores at day 3 after DNBS. DNBS increased MPO activity from 0.5 ± 0.13 U/mg in control mice to 1.74 ± 0.39 U/mg. As shown in Table 1, VXP resulted in significantly higher MPO activity of 4.72 ± 0.75 U/mg compared with sham-operated mice. SAP levels increased from 45 ± 5 g/mL in DSS–sham-operated mice to 198 ± 12 g/mL in DNBS vagotomized mice (Table 1). VXP significantly increased the severity of colitis, with histologic scores increasing from 5.68 ± 0.52 to 8.55 ± 0.58. In addition, we found significantly greater fold increases in the levels of TNF-α and IL-1β in the colon of DNBS-treated mice with VXP compared with sham-operated mice (Table 1).
Discussion

This study was prompted by the recent demonstration of a macrophage-mediated vagal reflex that attenuated inflammation immediately after systemic exposure of mice to lipopolysaccharides. The present study identifies a vagal cholinergic pathway that attenuates the inflammatory response over 5 days during experimental colitis. Colitis induced by 5% DSS was more severe in vagotomized mice and could be mimicked by administration of hexamethonium, but we cannot exclude an effect of reduced blood flow to the worsening of colitis seen in hexamethonium-treated mice. Conversely, administration of nicotine attenuated the deleterious effect of vagotomy in mice with colitis and in sham-operated mice with colitis. The absence of a protective role of the vagus in op/op mice with colitis implicated a role for M-CSF–derived macrophages in this anti-inflammatory reflex. Taken together, these findings extend the influence of the inflammatory Reflex to intestinal inflammation.

We used DSS (dissolved in the drinking water) and any difference in the inflammatory response after colitis could be attributed to consequences of surgery resulting in changes in the intake or delivery of DSS to the gut. It is therefore important to emphasize that no significant differences were seen in water intake between the sham-operated and vagotomized mice, and that the pyloroplasty overcame the problem of gastric retention of DSS after vagotomy. In addition, incompleteness of the vagotomy could have confounded results and this was assessed both histologically and functionally using CCK-8 challenge; mice in which vagotomy was considered incomplete were excluded from the experiments.

Our results show a protective role of the vagus over a 5-day period of inflammation with DSS. The onset of inflammation occurred more rapidly in vagotomized mice, implying a role for the vagus in attenuating the early events of the inflammatory cascade. This most likely is mediated via suppression of proinflammatory cytokine release from macrophages as part of the initial innate immune response to DSS. It also is possible that vagotomy altered mucosal barrier function, enhancing the exposure of the gut to DSS and other luminal factors such as bacterial antigens; previous studies have shown that intestinal permeability is modulated by cholinergic nerves and that vagotomy increases permeability in rat intestines. We showed the same profile of results in mice with DNBS colitis, indicating a broader applicability of our findings.

The present study builds on the findings of Mazelin et al who showed that selective inhibition of vagal afferent fibers, using perivagal application of capsaicin, worsened hapten-induced colitis in the rat. Our study used bilateral truncal vagotomy to remove both afferent and efferent fibers because it has been shown that electrical stimulation of the vagus attenuates cytokine production in the periphery. Furthermore, the present study identifies the macrophage as a critical cell in

Figure 2. (A) Histologic scores in mice 5 days post-DSS colitis and in mice without colitis. Five days after colitis, VXP increased the histologic score (n = 12) and nicotinic (Nico) treatment (20 μg/mL) decreased the score significantly (n = 10). Hexamethonium (Hex) (10 mg/kg, SC) increases the score in the sham-operated group significantly (n = 8) and nicotine tends to decrease it (n = 6). +P < .05 vs sham-DSS treated. ++P < .05. (B) VXP increases the score in op/7 mice (n = 9), but not in op/op mice (n = 9). The values are shown as means ± SD. *P < .05 vs op/7 sham-DSS treated. **P < .05. (A and B) Sham; ■, VXP. (C) Appearance of a control colon in a DSS-treated mouse, associated with one-third loss and shortening of crypts, a small mucosal erosion, mild inflammatory cell infiltration, and mild goblet cell depletion. (D) and in a vagotomized mouse higher areas of erosion are associated with more inflammatory cell infiltration. H&E staining: magnification, 10x.
mediating the anti-inflammatory effect of the vagus during intestinal inflammation. We used mice deficient in M-CSF to elucidate the role of macrophages in the anti-inflammatory role of the vagus nerve. The M-CSF–deficient op/op mouse has reduced numbers of circulating and tissue-based macrophages, which also are limited in terms of their abilities to differentiate and proliferate.2,37–39 Macrophages are considered to be important in the complete expression of colitis induced by DSS22 and our findings support this as we show a reduction in the severity of colitis in op/op mice compared with op/? mice exposed to DSS. Nevertheless, we found a significant degree of inflammation in DSS-treated op/op mice compared with control op/op mice, and this degree of inflammation would have been sufficient to identify a further worsening of colitis after vagotomy. We therefore interpret the absence of any worsening of the colitis in vagotomized DSS-treated mice to reflect a critical role for macrophages in the protection against inflammation conferred by the vagus nerve.

It is conceivable that other factors contribute to the protective effect of the vagus nerve in our study. For example, it is known that the vagus influences lymphocyte trafficking40 and mast cell numbers in the gut.41 In addition, the vagus influences gastrointestinal motility and controls the ileocecal valve in several species.42,43 However, we consider it unlikely that these mechanisms made a significant contribution to our findings because they are not known to be macrophage dependent and

Figure 3. Effect of VXP on colonic cytokine level by enzyme-linked immunosorbent assay measurements. VXP results in significant increases in DSS-treated mice of all 3 cytokines (n = 12). Nicotinic (Nico) treatment (20 μg/mL) decreases the cytokines significantly (n = 10). Hexamethonium (Hex) (10 mg/kg, SC) shows a significant increase of all 3 cytokines in sham-operated DSS-treated mice (n = 8). Nicotine induces a significant decrease of 2 cytokines in sham-operated DSS-treated mice (n = 6). Vagotomy increases levels in op/? mice (n = 9), but not in op/op mice (n = 9). +P < .05 vs sham-DSS treated. *P < .05 vs VXP-DSS treated. #P < .05 vs op/? sham-DSS treated.

Figure 4. Macroscopic score, histologic score, MPO activity, and SAP level in op/op and op/? mice with or without treatment with DSS 5%. All 4 markers are increased significantly after DSS 5% challenge (n = 6). The values are shown as means ± SD. Water treatment; □, DSS 5% treatment. *P < .05 compared with op/op DSS 5% treated.
we saw no significant difference in the inflammatory response between sham-operated and vagotomized op/op mice.

The direct correlate of our findings is unclear. Vagotomy seldom is performed these days and we could find no study of the natural history of IBD in patients who had undergone vagotomy for other reasons. In a long-term follow-up evaluation of patients with duodenal Crohn’s disease, vagotomy was associated with disease recurrence but it could not be ascertained whether this was owing to mechanical factors such as poor gastric drainage, or to a more aggressive inflammatory response.44 Histologic and functional studies have shown evidence of parasympathetic impairment in IBD. Necrosis of autonomic nerves has been documented in Crohn’s disease9 and is supported by functional studies showing autonomic imbalance in patients with ulcerative colitis45 and Crohn’s diseases,10 with evidence of parasympathetic impairment in both conditions. Interestingly, in Crohn’s disease this was associated with a trend toward more surgical interventions, implying a more aggressive form of the disease.10

It has been reported that in IBD smoking exacerbates inflammation in Crohn’s disease but is inhibitory in UC.46 In our 2 models of colitis we found that the lack of acetylcholine

Table 1. Influence of VXP on Macroscopic Score, MPO Activity, SAP Level, Histology Score, and Cytokine Level in Colonic Tissue After 3 Days of DNBS-Induced Colitis

<table>
<thead>
<tr>
<th></th>
<th>Sham mice</th>
<th>VXP mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline 4%</td>
<td>Ethanol 4% + saline 4%</td>
</tr>
<tr>
<td></td>
<td>DNBS 4% + ethanol 4%</td>
<td>Ethanol 30% + DNBS 4% + ethanol 30%</td>
</tr>
<tr>
<td>Macroscopic score</td>
<td>0.12 ± 0.11</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>MPO, U/mg/tissue</td>
<td>0.14 ± 0.62</td>
<td>0.5 ± 0.13</td>
</tr>
<tr>
<td>SAP, μg/mL serum</td>
<td>15 ± 2.5</td>
<td>45 ± 5</td>
</tr>
<tr>
<td>Histologic score</td>
<td>0</td>
<td>2.33 ± 0.45</td>
</tr>
<tr>
<td>II-1, fold change</td>
<td>2.6 ± 0.2</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>TNF-α, fold change</td>
<td>3.9 ± 0.3</td>
<td>4.8 ± 0.6</td>
</tr>
</tbody>
</table>

NOTE. Values are shown as means ± SD.

—, less than the lower standard detection.

*P < .05, n = 7, vs sham-operated DNBS treatment.

**P < .05, n = 7, vs sham-operated ethanol treatment.

***P < .05, n = 7, vs VXP ethanol treatment.
action on the nicotinic receptor is probably a source of colitis development, although in human disease nicotine can have 2 different effects: it can ameliorate or worsen the colitis in UC and Crohn’s disease patients. This last deleterious effect could come from detrimental effects of other components, not present in our 2 models but present when smoking.

This study, taken in conjunction with others, offers new therapeutic approaches to the management of IBD. The vagus has been shown to exert its anti-inflammatory effect via the release of acetylcholine, which interacts with nicotinic receptors on macrophages to inhibit the release of proinflammatory cytokines.19 The α 7 subunit of the nicotinic acetylcholine receptor is critical for this action, which results in the selective suppression of proinflammatory cytokine secretion from macrophages.18 de Jonge et al17 showed that the vagal anti-inflammatory pathway acts by α 7 subunit–mediated Jak2-STAT3 activation. Thus, agonists that preferentially interact with the α 7 subunit of this receptor may be useful in the management of IBD. In addition, recent work has shown that dietary fat can attenuate inflammation via the release of CCK, which acts on vagal afferents to suppress cytokine release. Thus, the protective effect of the vagus also can be harnessed by dietary manipulation in the treatment of inflammatory disease.48

References


Received November 29, 2005. Accepted June 21, 2006.

Address requests for reprints to: Stephen M. Collins, MD, Room 4W8, McMaster University Medical Center, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. e-mail: scollins@mcmaster.ca; fax: (905) 521-4956.

Supported by grants from the Crohn’s and Colitis Foundation of Canada (to S.M.C.) and by a Canadian Association of Gastroenterology fellowship and l’Institut de France (to J.E.G.).

All the authors declare that no potential competing interests exist. The authors thank Amanda L. Begora for her expertise and help in the cholecystokinin octapeptide experiments.