Vagus nerve integrity and experimental colitis

Jean-Eric Ghia, Patricia Blennerhassett, and Stephen M. Collins

Intestinal Diseases Research Programme, Health Science Center, McMaster University, Hamilton, Ontario, Canada

Submitted 23 February 2007; accepted in final form 20 June 2007

Ghia J-E, Blennerhassett P, Collins SM. Vagus nerve integrity and experimental colitis. Am J Physiol Gastrointest Liver Physiol 293: G560–G567, 2007. First published June 21, 2007; doi:10.1152/ajpgi.00098.2007.—Previous studies have identified a counterinflammatory vagal reflex in the context of endotoxic shock. We have extended this observation to show that the vagus confers protection against acute (5 days) colitis induced by dextran sodium sulfate (DSS) or dinitrobenzene sulfonic acid (DNBS). We have shown that this is mediated via macrophages and involves the suppression of proinflammatory cytokines. In this study, we have examined whether the vagal integrity confers long-lasting protection by studying DNBS- and DSS-induced inflammatory responses in the colon at 9 to 61 days postvagotomy. The integrity of vagotomy was confirmed at all time points using CCK-induced satiety. As previously described in a DNBS and DSS model, vagotomy associated with the pyloroplasty increased all indices of inflammation. Vagotomy increased the disease activity index as well as the macroscopic and histological scores by 75 and 41%, respectively. In addition, myeloperoxidase (MPO) activity, serum levels of C-reactive protein (CRP), and colonic tissue levels of proinflammatory cytokine increased when colitis was induced 9 days postvagotomy. However, these increases in inflammatory indices were substantially diminished in mice with colitis induced 21, 33, and 61 days postvagotomy. This was accompanied by a decreased production of interleukin-10, transforming growth factor-β, Forkhead Box P3 (FOXP3) staining in colonic tissue, and serum corticosterone. These findings indicate that although vagal integrity is an important protective factor, other counterinflammatory mechanisms come into play if vagal integrity is compromised beyond 2 wk.

INFLAMMATORY BOWEL DISEASE (IBD) is a chronic intestinal inflammatory state and is considered the consequence of an aberrant immune response to luminal antigens (21). Tissue injury results from the release of inflammatory mediators, including acid metabolites and proinflammatory cytokines (41, 43, 48). In particular, increased secretion of proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) (5, 20), are considered to be important for the exacerbation of IBD and constitute targets for therapy. Cytokine production can be modulated by neurotransmitters, including those of the autonomic nervous system (31). 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 (18). Up to 35% of patients with ulcerative colitis exhibit autonomic imbalance with impaired parasymptathetic function, resulting in sympathetic dominance (29). Studies in animal models support the notion that autonomic imbalance contributes to the inflammatory drive in IBD. This is based on observations that the sympathectomy improves experimental colitis (33) and that administration of the parasympathomimetic nicotine improves colitis in animal models (19).

Previously, prompted by the demonstration of a vagally mediated anti-inflammatory reflex in an acute endotoxic shock model by Tracey and colleagues (7), we showed that the parasympathetic nervous system plays a counterinflammatory role in acute colitis induced by either dextran sodium sulfate (DSS) or dinitrobenzene sulfonic acid (DNBS) evident at 10 days after recovery (recovery time) between the vagotomy and pyloroplasty (VXP) and the induction of the colitis (24). In this model, we proposed that the presence of proinflammatory cytokines in the periphery is detected by vagal afferents, resulting in a vagal efferent response associated by an attenuation of cytokine release from macrophages. Via nicotinic acetylcholine (ACh) receptors (6, 52), it has also been shown that vagotomy, which accelerates LPS-induced septic shock and increases systemic TNF-α production, can enhance severity of experimental pancreatitis in mice (53) and that selective inhibition of vagal afferent fibers aggravates hapten-induced colitis (32). Conversely, stimulation of the vagus nerve selectively downregulated production of proinflammatory cytokines (54, 55). This vagal reflex involves the release of ACh that interacts with the α-7 subunit nicotinic receptor on macrophages (56). This is further supported by demonstrations in vitro that nico- tinic receptors are involved in the selective downregulation of LPS-induced release of TNF-α, IL-6, and IL-1β in cultured macrophages (7) and by the major role played by the macrophage population in the context of inflammation induced by DSS in vagotomized mice (24).

As the central nervous system (CNS) receives sensory input from the immune system through both humoral and neuronal routes, we can speculate that vagotomy will ultimately induce compensatory changes in the CNS, resulting in the activation of different pathways to contain the inflammatory response in the gut through the production of anti-inflammatory cytokine or corticosterone. Modification of the CD4+ /CD25+ /Forkhead box P3 (FOXP3) T regulatory (Treg) cell subpopulation directly in situ in the tissue can not be excluded. Transforming growth factor-β (TGF-β) and IL-10 (30) are pivotal immunoregulatory cytokines and can modify the T cell profile to Treg cells, which impact both the innate and the cell-mediated branches of the immune system. Studies using IL-10 knockout mice provide compelling evidence for the role of this cytokine in maintaining the gastrointestinal homeostasis. Similarly, studies have demonstrated multiple immunoregulatory effects of TGF-β, including T cell inhibition, B cell proliferation, and cytokine production. Both cytokines can inhibit the principal functions of monocytes and macrophages populations (22, 39, 46) and reduce the synthesis of IL-β, TNF-α...
synthesis by the monocytes (15, 22, 23, 46), and granulocyte activation (37). Activation of the adrenocorticotropic hormone-glucocorticoid axis after endotoxin administration (57) provided evidence that inflammatory stimuli can activate an anti-inflammatory signal from the CNS. Cytokine from the periphery can activate the hypothalamic-pituitary release of glucocorticoids and suppress further cytokine synthesis (52).

The existence of counterinflammatory vagal-dependent mechanisms has been demonstrated only in the short term following vagotomy and it is not known whether this persists or is eventually replaced by other mechanisms. In the current study, we have examined these possibilities by studying colitis induced by DSS or DNBS at long time intervals postvagotomy. Our results indeed indicate that the protective effect of the vagus does ultimately fade and that compensatory changes emerge to contain the inflammatory response.

MATERIALS AND METHODS

Animals. Male C57BL/6 (7–9 wk old) were purchased from Harlan Animal Suppliers (Indianapolis, IN) and maintained in the animal care facility at McMaster University under specific pathogen-free conditions. Mice were housed under standard conditions for a minimum of 1 wk before experimentation. All experiments were approved by the McMaster University animal ethics committee and conducted under the Canadian guidelines for animal research.

Vagotomy. Mice were anesthetized using ketamine (150 mg/kg ip) and xylazine (10 mg/kg ip), and ventral and dorsal truncal branches of the subdiaphragmatic vagus were cut (1 cm above gastroesophageal junction). Preliminary studies showed marked gastric dilatation in vagotomized mice, and a surgical pyloroplasty was therefore incorporated into the protocol. VXP were subsequently 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, and a pyloroplasty was performed. All mice were maintained on normal diet.

Validation of vagotomy. The ability of cholecystokinin to reduce food intake is completely dependent on the integrity of the vagus nerve (28, 47). To determine the functional integrity of vagotomy in our study, the different subgroup of mice received 40 μg/kg iv of cholecystokinin octapeptide (CCK-8, Sigma) at 8, 20, 32, and 60 days after VXP or sham surgery, and food intake was measured over 24 h. Functional integrity of VXP was ascertained by the absence of a CCK-induced suppression of feeding. The completeness of vagotomy was verified during postmortem inspection of vagal nerve endings using microscopical inspection.

Induction of DSS and DNBS colitis. One day after the end of the CCK-8 experiment, DSS (molecular mass 40 kDa; ICN Biomedicals, Aurora, OH) was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days (24). 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). A 10-cm PE-90 tubing (Clay Adam, Parsippany, NJ), attached to a tuberculin syringe, was inserted 3.5 cm into the colon. Colitis was induced by administering 100 μl of 4 mg of DNBS solution (ICN Biomedicals) in 30% ethanol (50%). Control mice (without colitis) received saline administration. Mice with colitis were supplied with 6% sucrose in drinking water to prevent dehydration.

Assessment of the severity of colitis: DAI. Disease activity index (DAI) scores have historically correlated well with the pathological findings in a DSS-induced model of IBD (13). DAI is the combined score of weight loss, stool consistency, and bleeding. Scores were defined as follows. Weight: 0, no loss; 1, 5–10%; 2, 10–15%; 3, 15–20%; and 4, 20% weight loss; stool: 0, normal; 2, loose stool; and 4, diarrhea; and bleeding: 0, no blood; 2, presence (Hemocult II “positive”, Beckman Coulter); and 4, gross blood. DAI was scored from day 0 to day 5 during DSS treatment.

C-reactive protein, IL-10, TGF-β1, and corticosterone assay in serum. Blood was collected 3 or 5 days after the beginning of the DNBS or DSS treatment, respectively, by intracardiac puncture in anesthetized (enflurane) mice. C-reactive protein (CRP), active TGF-β1, IL-10, and corticosterone levels were determined using ELISA commercial kit (Quantikine M murine; R&D Systems, Minneapolis, MN).

Macroscopic scores. Five days after the beginning of the DSS or 3 days after the beginning of the DNBS treatment, 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 immediately assessed. Macroscopic scores were performed using a previously described scoring system for DSS colitis (13) and for DNBS (3).

Colon histology and MPO activity. Formalin-fixed colon segments were paraffin-embedded and 3-μm sections were stained with hematoxylin and eosin. Colonic damage was scored based on a published scoring system that considers architectural derangements, goblet cell depletion, edema/ulceration, and degree of inflammatory cell infiltration (13). Myeloperoxidase (MPO) activity was determined following an established protocol (8). Briefly, MPO activity, used as a marker of neutrophil infiltration, was extracted, and the activity was measured using a modified version of the method described by Bradley et al. (9). Tissue samples were homogenized (50 mg/ml) in ice-cold 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammoniumbromide (Sigma). The homogenate was freeze-thawed three times, briefly sonicated, and then centrifuged at 12,000 rpm for 12 min at 4°C. The supernatant was then 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 quantity of enzyme able to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature.

Cytokine tissue levels. The colonic sample was homogenized in 700 μl of Tris–HCl buffer containing protease inhibitors (Sigma). Samples were centrifuged for 30 min, and the supernatant was frozen at 80°C until assay. Cytokine levels (IL-1β, IL-6, TNF-α, TGF-β1, and IL-10) and corticosterone were determined using an ELISA commercial kit (Quantikine M murine; R&D Systems, Minneapolis, MN). Results are presented as the fold time difference vs. control group without vagotomy under DSS treatment.

Immunohistochemistry. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded samples sectioned at 3 μm. Serial sections were deparaffinized in xylene (Fisher Scientific, Nepean, Ontario, Canada) and rehydrated through a graded series of ethyl alcohol and PBS. Endogenous peroxide was blocked by incubation in peroxidase-blocking reagent (DakoCytonumption, Mississauga, Ontario, Canada) for 30 min. After being washed with PBS, sections were predigested with proteinase K solution (DakoCytonumption) for 10 min at room temperature. After being washed and blocked of nonspecific binding with 5% bovine serum albumin in PBS for 25 min, the sections were incubated with polyclonal antibody against the COOH terminus of the FOXP3 protein (ab10563, dilution 1:50). After being washed, sections were incubated with envision [horseradish peroxidase (HRP)-coupled anti-rabbit secondary reagent; DakoCytonumption] for 30 min. The sections were developed with 3,3′-diaminobenzidine solution as chromogen (Sigma). The sections were counterstained with Meyer’s hematoxylin (DakoCytonumption), dehydrated, cleared, and mounted.

FOXP3-positive cells were counted in five different positions in each section that were chosen randomly. All immunohistochemical evaluations were performed in a blinded manner. Tonsillar tissue with follicular hyperplasia served as positive controls, displaying scattered T cells in the interfollicular area with nuclear expression of FOXP3. Negative controls were performed by omitting the primary antibodies.
Statistical analysis. Results are presented as means ± SE. Statistical analysis was performed using one-way ANOVA followed by the Student-Newman-Keuls multiple comparisons post hoc analysis and a P value of <0.05 considered significant.

RESULTS

Responses to CCK-8 and validation of vagotomy. All mice were tested after 8 days of recovery time (RT), and subgroups were tested at 20, 32, and 60 days after surgery. Food intake was significantly decreased by 80.9 ± 1.4% following CCK-8 injection in sham-operated mice compared with VXP mice +1.3 ± 0.9% after 8 days (Fig. 1A). Those VXP mice in which CCK induced a significant reduction in food intake 8 days after surgery were excluded from subsequent studies, on the assumption that the vagotomy was incomplete. After 20, 32, and 60 days, no significant differences were found in food intake, and water intake was not different between VXP and sham-operated mice (5.1 ± 0.9 and 5.9 ± 0.8 ml/24 h, respectively) (Fig. 1A).

Effect of VXP without colitis. VXP caused no changes in weight gain, colonic appearance, histology, CRP, MPO, or cytokine levels in mice without colitis. TNF-α, TGF-β, IL-1β, IL-10, IL-6, and corticosterone colonic tissue levels were below the lowest standard of the assay in these mice.

Effect of extension of recovery time after VXP on DSS-induced colitis. The extension of RT on sham-operated mice treated with DSS did not show any significant difference for all the markers (data not shown). Therefore, only one control group for each marker is presented. As previously described, VXP increased all the different inflammatory markers (24). DSS induced a colitis characterized by weight loss and frequent stools; this was evident by day 3 in sham-operated mice (Fig. 1B). In VXP mice, the onset of colitis was accelerated as well as the severity of injuries (reflected in the DAI) and were seen within 1 day of DSS. 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. However, these differences decreased with the extension of the RT and no significant change in DAI was seen within 61 days of VXP. It should be noted that there was still a significant increase of DAI during the 2 first days of colitis induced 33 days after VXP (Fig. 1B).

VXP significantly increased the macroscopic scores after 9 days of RT and 5 days of DSS treatment. Conversely, the extension of the RT significantly decreased the macroscopic scores, and no significant changes were seen within 33 and 61 days of RT compared with the sham-operated mice treated with DSS (Fig. 1C).

As shown in Fig. 2A, VXP significantly increased the severity of colitis with histological scores increasing from 2.1 ± 0.3 to 3.3 ± 0.1. This was associated with a greater loss of tissue architecture, edema, and a massive, mixed immune cell infiltrate (mononuclear cells, neutrophils, and eosinophils). The increase of RT within 33 and 61 days was associated with a decrease of the histological score. This profile is well correlated with the MPO activity while the extension of the RT decreased significantly the MPO activity, and no significant changes were seen within 33 and 61 days of RT compared with the sham-operated mice treated with DSS (Fig. 2B).

CRP levels increased for DSS-treated mice with VXP (Fig. 2C), and an extension of the RT decreased significantly the CRP level with no significant changes seen after 33 and 61 days of RT.
days of RT compared with the sham-operated mice treated with DSS.

In addition, we found a significantly greater fold increase in the levels of IL-1β and TNF-α in colonic tissue of DSS-treated mice with VXP, associated with a short RT, compared with a long RT (Fig. 3A). However, IL-6 showed a bivalent effect associated with a significant increase of the level during a short RT, followed by a decrease after 21 days of RT and associated with a rebound within 61 days of RT (Fig. 3A). IL-10, TGF-β, and corticosterone increased in the serum and in the tissue; this increase was associated with a longer RT (Fig. 3, A and B).

Effect of extension of recovery time after VXP on DNBS-induced colitis. VXP increased all the different inflammatory markers in the DNBS model of colitis (24). As shown in Table 1, extension of the RT decreased significantly the macroscopic scores, histological scores, the MPO activity, and the CRP level in DNBS-vagotomized mice. In addition, we found a significant decrease of IL-1β and TNF-α levels in the colon of DNBS-treated mice with VXP, associated with a longer RT. Conversely, a significant increase of IL-10, TGF-β, and corticosterone in the colonic tissue was associated with a longer RT. As mentioned in the DSS-model, IL-6 showed the

Fig. 3. A: effect of VXP on colonic cytokine level by ELISA measurements. Extension of the RT in VXP-mice resulted in a significant decrease of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) and an increase of IL-10, transforming growth factor-β (TGF-β), and corticosterone (Cortico) levels in DSS-treated mice (*P < 0.05, n = 12). Extension of the RT within 33 and 61 days decreased level of CRP compared with VXP group. Values are shown as means ± SE.
same bivalent effect (Table 1). Extension of the RT increased significantly the level of IL-10, TGF-β, and corticosterone in the serum (Table 2).

To elucidate the role of regulatory T cells, we used a staining based on the FOX3 marker, present in Treg cells. Control mucosa with no treatment contained only very few FOX3+ cells in the LP (data not shown). A FOX3 immunohistochemistry did not show a significant increase of Treg cells in the sham-operated mice 9 days post-VXP and progressively decreased until it reached the control experiments within 61 days of RT. The onset of inflammation occurred more rapidly in vagotomized mice 9 days post-VXP and progressively decreased until it reached the control experiments within 61 days of RT. The post-vagotomy interval can affect the levels of proinflammatory cytokines (7). In our study, we found that the extension of the RT increased the short term post-VXP and during acute inflammation in two models of colitis (24). The present study indicates that this protective effect of the vagus does not persist indefinitely. Colitis induced by 5% DSS or by 4 mg DNBS was less severe in vagotomized mice with a prolonged interval post-VXP. Proinflammatory cytokines were significantly decreased and production of anti-inflammatory cytokines, corticosterone, and Treg cells was upregulated, attenuating the deleterious effect of vagotomy in mice with an extensive RT. Taken together, these findings extend the influence of the inflammatory reflex beyond those early time points identified in the current literature (52), but that protection ultimately fades and is replaced by other counterinflammatory influences.

We used DSS (dissolved in the drinking water) to induce colitis, and any difference in the inflammatory response following colitis might 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 over time and that the pyloroplasty overcame the problem of gastric retention of DSS following vagotomy. In addition, incompleteness of the vagotomy could have confounded results, and this was assessed functionally using CCK-8 challenge; mice in which vagotomy was considered incomplete at the first time of experiment completed 8 days after surgery were excluded from the experiments. In the remaining mice the failure of a CCK-8 challenge to reduce food intake at days 20, 32, and 60 indicates that the loss of counterinflammatory influences is not due to restoration of vagal integrity.

Our results demonstrate a protective role of the vagus over a 5-day period of inflammation with DSS and 3 days with DNBS. The onset of inflammation occurred more rapidly in vagotomized mice 9 days post-VXP and progressively decreased until it reached the control experiments within 61 days of VXP. This most likely reflects the loss of vagally mediated suppression of proinflammatory cytokine release (17). As previously described, proinflammatory cytokines are increased in the short term post-VXP and during acute inflammation induced by LPS (7). In our study, we found that the extension of the postvagotomy interval can affect the levels of proinflammatory cytokines like TNF-α and IL-1β present in the colonic tissue by decreasing them. Taking into consideration that the proinflammatory cytokines are mostly released by the macro-

Table 1. Influence of VXP and RT on macroscopic score, MPO activity, CRP level, histology score, and cytokine level in colonic tissue after 3 days DNBS-induced colitis

<table>
<thead>
<tr>
<th></th>
<th>Sham Mice</th>
<th>VXP Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ETH30%</td>
<td>DNBS4%</td>
</tr>
<tr>
<td>Macropscopic score</td>
<td>0.66±0.66</td>
<td>4.31±0.45</td>
</tr>
<tr>
<td>MPO U/mg tissue</td>
<td>0.66±0.1</td>
<td>4.3±0.45</td>
</tr>
<tr>
<td>CRP μg/ml serum</td>
<td>7.7±0.4</td>
<td>46.5±5.2</td>
</tr>
<tr>
<td>Histological score</td>
<td>2.5±0.6</td>
<td>2.5±0.5</td>
</tr>
<tr>
<td>IL-1β fold change</td>
<td>3.2±0.6†</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>TNF-α fold change</td>
<td>1.9±0.2‡</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>IL-6 fold change</td>
<td>2.1±0.4‡</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>IL-10 fold change</td>
<td>1.2±0.3‡</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>TGF-β fold change</td>
<td>3.1±0.2‡</td>
<td>3.1±0.2</td>
</tr>
<tr>
<td>Corticosterone fold change</td>
<td>1.2±0.4‡</td>
<td>1.2±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, n = 7, vs. sham DNBS treatment. †P < 0.05, n = 7, vs. sham ethanol treatment. ‡P < 0.05, n = 7, vs. VXP ethanol treatment.

Table 2. Influence of VXP and RT on cytokine level and corticosterone in serum after 3 days DNBS-induced colitis

<table>
<thead>
<tr>
<th></th>
<th>Sham Mice</th>
<th>VXP Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNBS4% + ETH30%</td>
<td>RT +9 DNBS4% + ETH30%</td>
</tr>
<tr>
<td>IL-10 fold change</td>
<td>2.1±0.2*</td>
<td>2.05±0.9†</td>
</tr>
<tr>
<td>TGF-β fold change</td>
<td>1.6±0.1†</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>Corticosterone fold change</td>
<td>2.1±0.3*</td>
<td>2.2±0.4†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, n = 7, vs. sham ethanol treatment. †P < 0.05, n = 7, vs. VXP ethanol treatment.
Inflammatory cytokine like TGF-β, as TGF-β can inhibit the IL-6-induced conversion of M1 cells at the intermediate stage of monocytic differentiation (34).

Levels of the immunoregulatory cytokine IL-10 and anti-inflammatory cytokine TGF-β were found to be significantly higher after an extension of the RT. Several studies have reported that both cytokines can inhibit the production of cytokines, chemokines, and prostaglandin synthesis by LPS-stimulated neutrophils and in DSS colitis (10, 36, 38, 51). Moreover, the transgene protein IL-10 was shown to markedly inhibit endotoxin-induced TNF-α production by mouse and rat macrophages in vitro (58). Furthermore, administration of a recombinant TGF-β decreases TNF-α in several models of inflammation, as well as in clinical trials (12). Conversely, inhibition of IL-10 augments tissue damage in vivo and the production of TNF-α and IL-1 in vitro (51). IL-10 is also central in the ability of Treg cells to inhibit colitis (4, 45), and some results suggest that Treg cells controlling intestinal inflammation are TGF-β dependent (44). In our study, we show that FOXP3 staining is increased within 61 days of VXP, indicating a potential relationship among IL-10, TGF-β, and Treg cells. Studies in mouse models of experimental colitis have demonstrated that intestinal inflammation not only can be prevented but also can be cured by the presence of CD4+CD25+FOX3 T cell associated with regulatory properties in the gut mucosa (16, 35). Therefore, an increased production of IL-10 and TGF-β in the colon and in the serum of vagotomized mice associated with a long RT may contribute to a lower degree of inflammation, a result observed in the present study.

Interestingly, we found changes not only in the cytokine pathways, but also in the hypothalamic-pituitary-adrenal (HPA) axis in our models post-VXP. The levels of corticosterone paralleled those of IL-10 and TGF-β in our study. To compensate for VXP, the HPA axis appeared to develop a stronger countereffect, as reflected by the corticosterone response. It has been shown that hydrocorticosterone can limit the level of circulating TNF resulting from an injection of LPS (42) and that corticoid can suppress inflammation in IBD patients (27).

Parasympathetic impairment results in a dominant sympathetic drive, and it is known that this enhances colonic inflammation (9). In addition, vagotomy alters lymphocyte trafficking (2) and mast cell numbers in the gut and influences in gut physiology (25), and these factors could contribute to the changes in severity of colitis seen in our study. In addition, the vagus influences gastrointestinal motility and controls the ileocecal valve in several species (14, 40) (11) and that vagotomy can inhibit the production of TNF-α (11) and that vagotomy increases permeability in rat intestine (26).

Whereas vagotomy is no longer a frequently performed procedure and there are no long-term studies of the natural history of IBD postvagotomy, our results indicate the importance of the neural regulation of intestinal inflammation by demonstrating the persistence of the protective vagal effect for several weeks in two models of colitis. Our results also illustrate that when this mechanism ultimately fades, compensatory
changes occur, and the nature of these changes is the basis of our ongoing work.

ACKNOWLEDGMENTS
We thank M. Patrick for expertise and help in CCK-8 experiments, and W. Jackson. All the authors declare that no potential competing interests exist.

GRANTS
This study was supported by grants from the Crohn’s and Colitis Foundation of Canada to S. M. Collins. J-E Ghia was supported by a Canadian Institute of Health Research team grant. J-E Ghia was supported by a Canadian Institutes of Health Research team grant. J-E Ghia was supported by a Canadian Institutes of Health Research team grant.

REFERENCES


