The protective effect of the vagus nerve in a murine model of chronic relapsing colitis

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Ghia J-E, Blenerhasset P, El-Sharkawy RT, Collins SM. The protective effect of the vagus nerve in a murine model of chronic relapsing colitis. Am J Physiol Gastrointest Liver Physiol 293: G711–G718, 2007. First published August 2, 2007; doi:10.1152/ajpgi.00240.2007.—The vagus nerve inhibits the response to systemic administration of endotoxin, and we have recently extended this observation to show that the vagus attenuates acute experimental colitis in mice. The purpose of the present study was to determine whether there is a tonic anti-inflammatory influence of the vagus on colitis maintained over several weeks. We assessed disease activity index, macroscopic and histological damage, myeloperoxidase (MPO) activity, and Th1 and Th2 cytokine profiles in chronic colitis induced by administration of dextran sodium sulfate (DSS) in drinking water for three cycles during 5 days 11 days of rest between each cycle (DSS 3, 2, 2%) in healthy and vagotomized C57BL/6 mice and in mice deficient in macrophage-colony stimulating factor (M-CSF). A pyloroplasty was performed in vagotomized mice. Vagotomy accelerated the onset and the severity of inflammation during the first and second but not the third cycle. Although macroscopic scores were not significantly changed, histological scores as well as MPO activity and colonic tissue levels of IL-1α, TNF-α, IFN-γ, and IL-18 but not IL-4 were significantly increased in vagotomized mice compared with sham-operated mice that received DSS. In control mice (without colitis), vagotomy per se did not affect any inflammatory marker. Vagotomy had no effect on the colitis in M-CSF-derived macrophage-deficient mice. These results indicate that the vagus protects against acute relapses on a background of chronic inflammation. Identification of the molecular mechanisms underlying the protective role of parasympathetic nerves opens a new therapeutic avenue for the treatment of acute relapses of chronic inflammatory bowel disease.

Vagotomy; chronic experimental colitis; inflammatory bowel disease; cytokine; macrophages

INFLAMMATORY BOWEL DISEASES (IBD), including ulcerative colitis and Crohn’s disease, are chronic inflammatory disorders of the gastrointestinal tract that result in significant morbidity. The pathogenesis of IBD is unknown but is thought to reflect an interaction of genetic and environmental factors; chronic inflammation results from immune dysregulation and an intolerance of commensal bacteria in the gut (25, 34, 35). Clinical observations also suggest that the nervous system influences the clinical course of IBD. This is supported by the beneficial effects of lidocaine (40) or nicotine (28) in at least a subset of IBD patients. More direct evidence of a neural modulation is from a case study in which spinal cord stimulation, a therapeutic strategy for treating neuropathic pain, induced relapses of ulcerative colitis (23).

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 (12). Up to 35% of patients with ulcerative colitis exhibit autonomic imbalance, with impaired parasympathetic function resulting in sympathetic dominance (24). Studies in animal models demonstrate that autonomic imbalance contributes to the inflammatory drive of experimental colitis. For example, sympathectomy improves experimental colitis (27) and administration of the parasympathomimetic nicotine improves colitis in animal models (13). Vagal modulation of endotoxemia shock (1) and attenuation of inflammation in a model of acute experimental colitis (20) is mediated via nicotinic ACh receptors on macrophages. This has raised the possibility of using selective agonists of this receptor to suppress inflammation in patients with IBD and other chronic inflammatory conditions (43). However, to date evidence of vagal protection against gut inflammation is restricted to acute inflammatory responses induced in mice without pre-existing inflammation and is not therefore strictly applicable to human IBD.

The dextran sodium sulfate (DSS) model, originally reported by Okayasu et al. (32), has been used extensively to investigate the role of the different cell types in colitis and macrophages, neutrophils, and monocytes (7, 11, 14, 42). This model is based on the oral administration of DSS in drinking water and can be used to study acute or chronic colitis by adjusting the concentration and duration of DSS administration (32).

In the present study, we have exploited the DSS model to determine whether the vagus nerve modulates a chronic inflammatory response in the murine colon. Our results show that the vagus nerve attenuates chronic colitis by a mechanism that involves macrophage-colony stimulating factor (M-CSF)-derived macrophages but that this protection is transient.

MATERIALS AND METHODS

Animals. Male C57BL/6 mice (7–9 wk old) were purchased from Taconic Animal Suppliers (Indianapolis, IN) and were maintained in the animal care facility at McMaster University under specific pathogen-free conditions. +/− breeding pairs were purchased from Jackson (Bar Harbor, ME). We used mice with a mutation in the gene encoding M-CSF; homozygotes (op/op) have a defect in osteoclast activity, are osteopetrotic, and lack a subset of macrophages (45, 49). Nonhomozygote mice (+/+ or +/op) were phenotypically indistinguishable and were used as controls (+/+), as previously described (18). Because osteopetrotic op/op mice lack teeth; they were fed a powdered diet, whereas +/− mice received conventional food. No differences in food intake or body weight were observed between

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these groups. 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
were conducted under the Canadian guidelines for animal research.

Vagotomy. Mice were anesthetized with 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 the gastroesopha-
geal junction). Preliminary studies showed marked gastric dilatation
in vagotomized mice, and a surgical pyloroplasty was therefore
incorporated into the protocol. Vagotomy (VX) with pyloroplasty (P)
was 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, but a
pyloroplasty was performed. All mice were maintained on normal
diet.

Validation of VX. The ability of cholecystokinin to reduce food
intake is completely dependent on the integrity of the vagus nerve (22,
36). To determine the functional integrity of VX in our study, mice
received 40 μg/kg of cholecystokinin octapeptide (CCK-8; Sigma) by
intravenous tail injection (4) 10 days after VX or sham surgery, and
food intake was measured over 24 h. The integrity of VX lasts well
beyond the time frame of the present studies, for as long as 62 days
(19). Functional integrity of VX 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 by
using a microscope.

Induction of DSS colitis. Two days after the end of the CCK-8
experiment, DSS (40 kDa; ICN Biomedicals, Aurora, OH) was added
to the drinking water in a final concentration of 3% (wt/vol) for 5
days. Then mice were transferred to water for 11 days. This cycle was
repeated twice more with 2% of DSS. Controls were all time matched
and consisted of mice that received normal drinking water only. Mean
DSS consumption was noted per cage each day.

Assessment of the severity of colitis: disease activity index. Disease
activity index (DAI) scores have historically correlated well with the
pathological findings in a DSS-induced model of IBD (8). 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
of 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
of

RESULTS

Responses to CCK-8. Food intake was significantly decreased by 78.7 ± 2.4% to 1.9 ± 2.3% following CCK-8 injection in sham-operated mice compared with VX mice (data not shown). Those VX mice in which CCK induced a significant reduction in food intake were excluded from subsequent studies, on the assumption that the VX was incom-
plete. Water intake was not different between VX and sham-
operated mice (5.7 ± 0.7 and 6.1 ± 0.6 ml/24 h, respectively).

Effect of VX without colitis. VX caused no changes in weight gain, colonic appearance or histology, MPO, or cyto-
kine levels in C57BL/6 and M-CSF mice without colitis.

The effect of VX on DSS-induced chronic colitis. DSS
induced a colitis characterized by weight loss and frequent
stools; this was evident by day 4 in sham-operated mice. In
VX mice, the onset of colitis and injury were accelerated,
reflected in the DAI, as seen within 2 days of DSS. As shown in
Fig. 1A, the DAI was significantly higher in VX mice
compared with the sham-operated mice on each of the 4 last
days of colitis during the first cycle; the differences between
groups reached statistical significance from day 2 up to day 9.
During the second cycle of DSS + water, significant differ-
ences between the two groups were seen only during the DSS
treatment phase. No significant differences were seen during
the third cycle. VX did not significantly increase the macro-
scopic scores after three cycles of DSS (1.6 ± 0.52 for sham-operated and 2 ± 0.29 for VX group; Fig. 1B). There
was a trend toward an increase in MPO activity in DSS-treated
sham-operated mice after three cycles of DSS (Fig. 1C). In
contrast, MPO activity was 1.17 ± 0.48 U/mg in sham-
operated mice and 3.93 ± 0.75 U/mg in VX mice (Fig. 2C).
As shown in Fig. 2, A and B, VX significantly increased the
severity of colitis, with histological scores increasing from
2.58 ± 0.15 to 3.45 ± 0.18. This was associated with greater
tissue damage and a large infiltrate of immune cells, including
mononuclear cells, neutrophils, and eosinophils. As shown in
Fig. 2B, collagen deposition, as reflected by Masson’s
trichrome staining, was significantly higher in the mucosa and
submucosa in the VX group compared with the sham-oper-
ated group. Suberosal focal bundles of collagen were found
more frequently in the VX mice compared with sham-oper-
ated controls.

Significantly greater increases were found in the levels of IL-1β
(5.9-fold), TNF-α (2.5-fold), INF-γ (1.63-fold), and
IL-18 (1.3-fold) in the colon of DSS-treated mice with VX
compared with sham-operated mice (Fig. 3, A–C and E). In
contrast, no significant changes in IL-4 were seen (Fig. 3D).
The effect of VXP on DSS-induced colitis on M-CSF-deficient mice. DSS induced a significant colitis in both olop and +/+ mice, although more inflammation was seen in the +/+ mice, as described previously (20). The olop mice exhibited a slower onset of DSS-induced colitis compared with +/+ mice (data not shown). In contrast to the increased severity of colitis seen in C57BL/6 mice with VXP, VX did not alter the severity of colitis in olop mice across all three cycles of DSS (Fig. 4A). Similarly, there was less macroscopic damage in olop mice and in +/+ mice with colitis compared with C57BL/6 mice, and this was not altered by VXP, as shown in Fig. 4B. Conversely, MPO activity was significantly increased after VXP in +/+ mice (0.75 ± 0.27 and 1.4 ± 0.14 U/mg, respectively; Fig. 4C), but VXP had no effect in olop mice (0.41 ± 0.48 and 0.8 ± 0.26 U/mg, respectively), as shown in Fig. 4C. Histological damage scores in DSS-treated olop mice were significantly decreased compared with +/+ mice, with scores decreasing from 2.31 ± 0.12 to 1.35 ± 0.31. As shown in Fig. 5, this pattern was not altered in olop VXP mice with DSS colitis. We found significantly greater increases in the levels of IL-1β (3.6-fold), TNF-α (2.3-fold), and INF-γ (1.5-fold) in the colon of +/+ DSS-treated mice with VXP, and this pattern was also evident in VXP olop mice with colitis (Fig. 6, A–C). No significant changes were seen for either IL-4 or IL-18 across any group (Fig. 6, D and E).
DISCUSSION

This study was prompted by the recent demonstration of a macrophage-mediated vagal reflex that attenuated inflammation during acute colitis (20) and extends that work by showing that the vagus attenuates the inflammatory response during a chronic model of experimental colitis. Inflammation induced by three cycles of DSS was more severe in vagotomized mice compared with control. The absence of a protective role of the vagus in op/op mice with colitis implicates a role for M-CSF-derived macrophages in this vagal inhibition. Together, these findings extend the relevance of the previously described inflammatory reflex (41) to a chronic model of intestinal inflammation.

We used DSS (dissolved in the drinking water), and any difference in the inflammatory response following colitis could be attributed to changes in the intake or delivery of DSS to the gut secondary to surgery. It is therefore important to emphasize that no significant differences were seen in water intake between the sham-operated and VXP mice and that the pyloroplasty overcame the problem of gastric retention of DSS following VX. In addition, incompleteness of the VX, which could have confounded results, was excluded from consideration because mice that exhibited a reduction in feeding following CCK-8 were removed from the study.

Our results demonstrate a protective role of the vagus over a 48-day period of inflammation induced by repeated exposure to DSS. The paradigm is one of acute on chronic inflammation, and our results suggest that the protective role of the vagus is more evident during the acute exacerbations that temporally correlate with DSS exposure. The onset of inflammation occurred more rapidly in VXP mice during the first cycle, 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 (10). A similar pattern was seen during the second cycle of
DSS. In contrast, indices of inflammation were similar in VXP and sham-treated mice during the intervals between DSS exposure. In addition to the suppression of proinflammatory cytokine production by macrophages, it is possible that VXP altered mucosal barrier function and colonic contractions during the two first cycles, enhancing the exposure of the gut to DSS and other luminal factors such as bacterial antigen, because previous studies have shown that intestinal permeability is modulated by cholinergic nerves (39) and that VX increases permeability in rat intestine (21). An effect of altered colonic contractility, altering exposure time to DSS, is not excluded, because this is regulated in part by ACh (44). In addition, we acknowledge that parasympathetic impairment results in a dominant sympathetic drive, which is known to enhance colonic inflammation (27).

Recent studies have shown that the protective effect of the vagus does ultimately fade and that compensatory changes emerge to contain the acute inflammatory response (19). These include the increased secretion of corticosteroid as well as the production of counterinflammatory cytokines (19). Cytokines released systemically during colitis may act directly on the brain to activate pathways that modify the inflammatory response in the periphery. For example, at the level of the area postrema (AP), IL-1β can act on endothelial cells to induce the synthesis of prostaglandins, which may act on neurons adjacent to the AP or directly on AP neurons connected to catecholamine neurons of the nucleus of the solitary tract (NTS). For example, IL-1β can activate A1 noradrenergic cells of the ventrolateral medulla, A2 noradrenergic cells of the NTS, and also C1 and C2 adrenergic cells of the ventrolateral medulla and the NTS, respectively. Stimulation of corticotropin-releasing factor cells within the medial parvocellular division of the paraventricular nucleus leads to the release of ACTH and initiates a glucocorticoid response that can protect the against potentially toxic effects of cytokine (5, 37). However, those observations occurred in the context of a primary acute inflammation, and extrapolation to the present paradigm of acute inflammation on chronic inflammation may not be justified. Thus the basis for loss of vagal protection beyond two cycles of DSS remains unclear.

Chronic colitis induced by DSS involves both a Th1 and a Th2 cytokine response, in addition to the production of IL-1β, TNF-α, INF-γ, IL-4, and IL-18 (37). We found that the worsening of inflammation in VXP mice was accompanied by a further increase in the Th1 cytokine INF-γ but not Th2 cytokine IL-4. Unlike IL-1β and TNF-α, which are produced mainly by macrophages (9), IL-4 is restricted to Th2 cells bearing the Ly-2 antigen and natural killer cells (6, 47) and is also produced by macrophages (16, 31). Whereas lymphocytic infiltration is a component of the chronic inflammatory response in this model, the effect of VX was more evident during the acute exacerbations of chronic inflammation, thus explaining the further increase in INF-γ but not IL-4.
The present study identifies the macrophage as a critical cell in 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 are also limited in terms of their abilities to differentiate and proliferate (17, 45, 46, 48). Macrophages are considered to be important in the complete expression of colitis induced by DSS (15), and our findings support this, because we show a reduction in severity of colitis in op/op mice compared with +/? mice exposed to chronic DSS during the three cycles (data not shown). Previously (20), 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 following VX. We therefore interpret the absence of any worsening of chronic colitis in M-CSF-deficient VX-DSS-treated mice to reflect a critical role for macrophages in the protection against inflammation conferred by the vagus nerve.

This study extends our understanding of the counterinflammatory influence of the vagus nerve from a context of acute to chronic inflammation, reminiscent of the natural history of IBD. Previous studies were restricted to the induction of acute inflammation in healthy mice (9, 26, 33). Our results show that the modulatory effect of the vagus is also evident on a background of chronic inflammation but that it expires over time. Nevertheless, these results do not necessarily mitigate against a role for parasympathomimetic drugs and agonists of the α7-nicotinic receptor subtype in IBD. On the basis of the results of this study, we propose...
that such agents would be more beneficial in managing acute relapses rather than controlling chronic inflammation in IBD, and particularly in those with autonomic imbalance and parasympathetic impairment (29, 40).

REFERENCES


