Reactivation of Inflammatory Bowel Disease in a Mouse Model of Depression

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See editorial on page 2058.

BACKGROUND & AIMS: Patients with inflammatory bowel disease (IBD) frequently also have depression, yet little is known of its role in IBD pathogenesis. We investigated whether the development of depression after the establishment of chronic inflammation reactivates an acute relapse of IBD and underlying pharmacologic mechanisms in mouse models.

METHODS: Colitis was induced by administration of dextran sulfate sodium (DSS) or dinitrobenzenesulfonic acid to C57BL/6 mice. Depression was induced by olfactory bulbectomy or chronic intracerebroventricular injection of reserpine. Colitis was reactivated by subsequent exposure to DSS or dinitrobenzenesulfonic acid. Some mice were given the antidepressant desmethylimipramine. Acute DSS-colitis was induced in mice lacking the α7 subunit of the nicotinic acetylcholine receptor (α7nAchR), and vagotomy was performed. Disease severity and colon tissue histology and inflammation were evaluated. Levels of C-reactive protein and proinflammatory cytokines were determined by enzyme-linked immunosorbent assay analysis of colon samples and macrophage culture.

RESULTS: Induction of depression reactivated inflammation in mice in which colitis had been established and become quiescent. The induction was associated with impaired cholinergic inhibition of proinflammatory cytokine secretion by macrophages and mediated by α7nAchR on these cells; macrophages isolated from depressed mice showed increased proinflammatory cytokine secretion. Depression-induced reactivation of colitis was prevented by desmethylimipramine and accompanied by a normalization of proinflammatory cytokine secretion.

CONCLUSIONS: Depression reactivates dormant chronic colitis via the α7nAchR. These findings encourage closer monitoring of behavior for signs of depression in IBD patients because treatment might prevent inflammatory conditions. Furthermore, α7nAchR agonists might achieve this effect without the need for psychotropic medication.

Inflammatory bowel diseases (IBD) are idiopathic, chronic, relapsing intestinal disorders of complex pathogenesis, which are represented mainly by Crohn’s disease (CD) and ulcerative colitis (UC). IBD is the most common and serious chronic inflammatory condition of the gastrointestinal tract and affects children and young adults in the prime of their lives and the incidence vary from 4 to 66 per 100,000 population of CD depending on the country.1 The present view is that the initiation of IBD is due to a convergence of a genetically determined susceptibility to inflammation and environmental triggers. Several factors are known to initiate relapses, including infection, ingestion of nonsteroidal anti-inflammatory drugs, stress, and nicotine. It has been shown that nicotine can exert 2 distinct effects in IBD: ameliorating or worsening the colitis in UC and CD patients.2 Recently, a clinical study demonstrated that relapse rates for CD ranged from 10% to 60% and 11% to 90% for UC.3

Depression results from a combination of genetic, environmental, and psychologic factors. Depression is associated with variety of medical conditions, which include irritable bowel syndromes, IBD, and also ischemic heart disease particularly in men.4 There are 2 broad hypotheses to explain this relationship, and they relate to inflammation or to autonomic imbalance. Major depression is strongly associated with increased levels of C-reactive protein (CRP) among men.5 Depression was also associated with increases in inflammation and coagulation factors in individuals who were free of cardiovascular disease, offering a stronger link between depression per se and susceptibility to inflammation.6 Indeed, some depressed patients exhibited increased tumor necrosis

Abbreviations used in this paper: Ach, acetylcholine; α7nAchR, alpha-7 nicotinic acetylcholine receptor; CC, choline-chloride; CD, Crohn’s disease; CRP, C-reactive protein; DAI, disease activity index; DMI, desmethylimipramine, DNBS, dinitrobenzene sulfonic acid; DSS, dextran sulfate sodium; INF, interferon; ICV, intracerebroventricular; IL, interleukin; LPS, lipopolysaccharide; MLA, methyllycaconitine; MOP, microosmotic pump; MPO, myeloperoxidase; Obx, bullectomy; RM, reserpinized mice; TCA, tricyclic antidepressant; UC, ulcerative colitis.

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factor-α level, which normalized upon treatment with antidepressants. It remains unclear as to whether this relationship reflects either a causal role for depression in susceptibility to inflammatory stimuli or common inflammation-based etiopathology. The second broad hypothesis is that depression results in autonomic imbalance, with impaired parasympathomimetic functions and a dominant sympathetic drive contributing to cardiac pathophysiology. The notion that depression is associated with parasympathetic dysfunction has led to the exploitation of vagal electrical stimulation as a novel treatment for refractory depression.

Depression may coexist with CD more often than expected by chance. In some studies, depression was unrelated to disease activity and, in some cases, actually predated the onset of CD. However, in other studies, depression correlated well with disease activity, suggesting that it is secondary to the disability imposed by CD. We postulate that depression influences the natural history of IBD. Recently, we have demonstrated that the vagus nerve provides tonic inhibition of acute inflammation in a murine model of colitis. We have also provided proof of concept that depressive-like behavior is associated with an exaggerated response to acute inflammatory stimuli in the gut via a vagal nerve-dependent pathway. Given the psychiatric comorbidity seen in IBD, we have investigated whether the development of depression after the establishment of chronic inflammation reactivates an acute relapse of IBD and have examined underlying pharmacologic mechanisms.

To achieve this, we used a lymphocyte-independent model of chronic colitis induced by oral administration of dextran sulfate sodium (DSS) and a lymphocyte-dependent model induced by intracolonic administration of 2,4-dinitrobenzenesulfonic acid (DNBS). We then induced depression using 2 established models: intracerebroventricular (ICV) reserpine treatment and bilateral olfactory bulbctomy. Thus, depression was induced on a background of quiescent inflammation and more closely resembling the natural history of chronic IBD. We also examined whether the reactivation involves changes in barrier function, as previously described with stress-induced reactivation of colitis. The recent demonstration of a tonic counter-inflammatory influence of the vagus nerve on experimental colitis mediated via the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR) provides a potential link between the cholinergic pathways via the nicotinic receptors, behavior, and gut inflammation. We also examined whether the vagus nerve, α7nAChR, and cytokine production by macrophages are involved. Of potential clinical importance, we investigated whether depression-induced reactivation could be prevented by antidepressants or by agonists of the nicotinic acetylcholine receptor.

**Materials and Methods**

**Animals**

Male C57BL/6 (7–9 weeks old) and α7nAChR-deficient mice (B6.12957-Chrna7tm1bay) breeding pairs were purchased from Taconic Farms Suppliers (Albany, NY) and Jackson Laboratories (Bar Harbor, ME) and maintained single housed in the animal care facility at McMaster University under specific pathogen-free conditions after surgery. All experiments were approved by the McMaster University Animal Ethics Committee and conducted under the Canadian guidelines for animal research.

**Induction of Chronic DSS and DNBS Colitis**

DSS (40 kilodaltons; MP Biomedicals, Soho, Ohio) was added to the drinking water in a final concentration of 5% (wt/vol) for 5 days then mice were given standard water for 11 days. Cycle was repeated twice with 3% DSS. In a second set, mice were anesthetized with isoflurane (Abbott, Toronto, Ontario, Canada). A 10-cm-long PE-90 tubing (Clay Adam, Parsippany, NJ), attached to a tuberculin syringe, was inserted (intrarectally) 3.5 cm into the colon. Colitis was induced by intrarectal administration of 100 μL of 4 mg of DNBS solution (ICN Biomedical Inc, Aurora, OH) in 30% ethanol, and the mice were left for 3 days. Control mice (without colitis) received saline administration. Mice with colitis were supplied with 6% sucrose in drinking water to prevent dehydration.

**Surgical Procedures and Reactivation of Colitis**

Forty-eight, 52, and 57 days after the induction of colitis by DSS or DNBS, microosmotic pumps (MOP) (Alzet, Cupertino, CA) were filled with reserpine solution (1 μg/day for 14 days) or vehicle, choline chloride (CC) (2 μg/day, 10 days), or methyllycaconitine (MLA) (5 μg/day, 5 days) (Sigma–Aldrich, Mississauga, Ontario, Canada), respectively, all dissolved in water except for reserpine diluted in (acetic acid 0.2%, pH balanced to 5). In another group, the MOP was placed directly in the peritoneum and was loaded with the same dose of reserpine or the vehicle. Bilateral ablation of the olfactory bulbs was performed using methods similar to those described previously. Next, colitis was reactivated on days 57 and 59 by DSS 2% or DNBS 2 mg. At the end of the experimental protocols, the brains of the mice were removed, and the completeness of olfactory bulb removal was verified by histology (data not shown). Vagotomy associated with a pyloroplasty and its assessment on acute colitis (DSS 5%) in α7nAChR-deficient mice were performed using methods similar to those described previously. Parts of the experimental protocols are described Figure 1.
Drugs

As previously described, desmethylimipramine (DMI) (Sigma–Aldrich) was administrated intraperitoneally (IP) at the dose of 15 mg/kg for 12 days starting 2 days postsurgery (day 50), nicotine or CC (20 μg/mL) were added to the drinking water on day 4 postsurgery (day 52), and hexamethonium or MLA (5 mg/kg) were administered by subcutaneous injection twice a day for 5 days post-DSS.

Tail Test

The immobility latency and the total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. This test is widely used in screening antidepressant drugs.

CRP and Cytokines Assays

Blood was collected, 5 or 3 days postreactivation associated with DSS or DNBS, respectively, by intracardiac puncture under Isoflurane anesthesia. Colonic sample were prepared as previously described, and CRP level, interleukin (IL)-1β, and IL-6 were determined using enzyme-linked immunosorbent assay commercial kit (R&D Systems, Minneapolis, MN).

Characterization of Inflammation

Disease activity index (DAI) and macroscopic scores and colonic damage were performed using a previously described scoring system for DSS colitis and for DNBS. Formalin-fixed colon segments coming from the splenic flexure were stained with H&E. Myeloperoxidase (MPO) activity was determined following an established protocol.

Isolation of Peritoneal Macrophages

Resident peritoneal cells were collected 5 or 3 days postreactivation associated with DSS or DNBS, respectively, as described with a slight modification. Cells were collected and resuspended in Dulbecco’s modified Eagle medium (DMEM) at 1 × 10^6 cells/mL. The overall cell viability was greater than 97%, and more than 94% of the cells were macrophages using DiffQuick (Jorgensen Laboratory, Loveland, CO) staining. Macrophage cultures were then exposed to CC (10^-6 mol/L) or to interferon (INF)-γ (150 IU/mL) (Sigma–Aldrich) where indicated. Ten minutes after the addition of the agents, lipopolysaccharide (LPS) (Sigma-Aldrich) was added to the cultures at a final concentration of 100 ng/mL. Supernatants were collected 3 hours after the addition of LPS.

Macrophage Staining

Five or 3 days postreactivation associated with DSS or DNBS treatment, respectively, F4/80 staining was performed using methods similar to those described previously. Briefly, sections were incubated with monoclonal rat anti-mouse antibody F4/80 (1:100, 18 hours at 4°C; Serotec, Raleigh, NC) then incubated with biotinylated goat anti-rat IgG antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour followed by horseradish peroxidase-conjugated streptavidin (1:300; DakoCytomation, Carpinteria, CA) for 30 minutes.
**Colonic Barrier Function**

Five days postreactivation with DSS, segments of the distal colon were mounted in Ussing chambers (WP Instruments, Longmont, CO), with a 0.6-cm² serosal tissue area exposed to oxygenated Krebs buffer maintained at 37°C, and colonic barrier function was assessed using methods similar to those described previously. Electrical current was measured in the voltage-clamp mode and expressed as short-circuit current (I_sc; μA/cm²). Tissue conductance (G; mS/cm²), which measures passive ion transport and represents paracellular barrier function, was assessed.

**Statistical Analysis**

Results are presented as means ± SEM. Statistical analysis was performed using 1- or 2-way ANOVA followed by the Tukey-Kramer multiple comparisons post hoc analysis, and a P value of < .05 was considered significant with n > 8 (KyPlot; KyensLab Inc, Soto Kando, Japan) (See Supplementary Materials and Methods).

**Results**

**The Induction and Resolution of Chronic Colitis**

In C57Bl/6 mice, acute administration of DSS 2% or 2 mg of DNBS have no effect on MPO activity or on CRP level and proinflammatory cytokines 5 and 3 days postinduction, respectively (data not shown). Chronic colitis was induced by 3 cycles of DSS 5%-3%-3%, 30 days after the beginning of the last cycle. The residual macroscopic score, MPO, CRP, IL-6, and IL-1β levels were 0.6 ± 0.2, 0.3 ± 0.1 U/mg, 17.4 ± 0.8 ng/mL, 0.9 ± 0.5 pg/mg, and 31.1 ± 8.4 pg/mg, respectively, compared with 0.9 ± 0.4, 0.5 ± 0.4 U/mg, 15.3 ± 2.8 ng/mL, 1.4 ± 0.8 pg/mg, and 37.2 ± 6.3 pg/mg, respectively, to the naïve group (P > .05). In a second set of mice, we used a single intrarectal administration of DNBS (4 mg) as another inflammatory stimulus, and this resulted in a severe colitis, characterized by extensive tissue damage and a large acute inflammatory cell infiltrate, with a macroscopic score of 3.7 ± 0.4 and a significant increase in MPO activity, from 0.7 ± 0.4 to 1.7 ± 0.3 U/mg after 3 days of treatment. These changes lasted several weeks, but the tissue appeared normal at day 62 (macroscopic score, 1.8 ± 0.5; MPO activity, 0.8 ± 0.3 U/mg; and IL-6 level, 3.4 ± 0.7 pg/mg compared with 2.1 ± 0.4, 1.3 ± 0.4 U/mg, 5.4 ± 1.8 pg/mg, respectively, to the naïve group).

**The Effect of Depression on Quiescent Colitis in Mice**

When mice were tested using the tail suspension test 8 days postsurgery, chronic ICV infusion of reserpine (1 μg/day, 14 days) significantly increased the duration of immobility and reduced the latency to immobility. Immobility duration and latency duration were 172.3 ± 11.8 and 27.8 ± 3.3 seconds in reserpinized mice (RM) group and 124.2 ± 5.1 and 62.5 ± 6.1 seconds in sham group, respectively (P < .05). RM showed a more severe reactivation of the colitis compared with vehicle mice (non-RM) treated or not with 2% DSS. Macroscopic scores, MPO, and CRP levels increased from 3.2 ± 0.3 to 6.2 ± 0.4, 2.7 ± 0.2 to 4.1 ± 0.3 U/mg, 29.1 ± 1.7 to 42.7 ± 2.1 ng/mL, respectively, following reactivation with 2% DSS and from 0.6 ± 0.2 to 2.9 ± 0.2, 0.83 ± 0.2 to 3.4 ± 0.5 U/mg, 17.4 ± 0.8 to 23.4 ± 0.7 ng/mL, respectively, in the absence of reactivation. Histologic score, IL-6, and IL-1β levels in colonic tissues were 71%, 46%, 66%, respectively, higher in RM-treated compared with non-RM treated with 2% DSS (Figure 2A and 2E) and 107%, 122%, and 243%, respectively, higher in the absence of reactivation (Figure 2A-E).

We next examined whether the effect of reserpine could be modulated by the treatment with antidepressant. DMI had no effect on any parameter of inflammation in mice with colitis that had not received reserpine (Figure 2). DMI therapy significantly improved the pa-
Chronic ICV Infusion of Reserpine Reactivates DNBS-Induced Colitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reactivation DNBS 2 mg</th>
<th>No Reactivation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>RM</td>
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<tr>
<td>Macroscopic score</td>
<td>3.1 ± 0.5</td>
<td>5.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>MPO U/mg of tissue</td>
<td>1.9 ± 0.3</td>
<td>3.1 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-6 pg/mg of protein</td>
<td>18.5 ± 2.6</td>
<td>34.5 ± 3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-1β pg/mg of protein</td>
<td>10.6 ± 0.8</td>
<td>13.9 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
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**NOTE.** The values are shown as means ± SEM. Influence of depressive-like behavior induced by reserpine (1 μg/day, 14 days), on macroscopic score, myeloperoxidase (MPO) activity, and cytokine level (IL-6 and IL-1β) in colonic tissue after reactivation or not with DNBS (2 mg in ethanol 30%).

<sup>a</sup><sup>P</sup> < .05, n > 8, vs sham-DNBS reactivated mice.
<sup>b</sup><sup>P</sup> < .05, n > 8, vs sham-DNBS nonreactivated mice.
<sup>c</sup><sup>P</sup> < .05, n > 8, vs reserpinized-DNBS nonreactivated mice.

Parameters of depressive-like behavior; immobility duration and latency duration were 100.2 ± 7.1 and 52.3 ± 3.1 seconds, respectively, in RM group and also reduced the vulnerability when treated or not with 2% DSS. Macrosopic scores, MPO, and CRP levels were significantly decreased to 2.7 ± 0.3, 2.6 ± 0.2 U/mg, 32.2 ± 2.1 ng/mL, respectively, in RM-DMI-treated compared with RM following reactivation with 2% DSS and to 0.8 ± 0.3, 1.1 ± 0.2 U/mg, 20.1 ± 0.4 ng/mL, respectively, without reactivation. Histologic score, IL-6, and IL-1β tissue levels were 34%, 98%, 46% lower in RM-DMI-treated compared with RM following reactivation with 2% DSS and 71%, 91%, and 80% lower without reactivation, respectively (Figure 2).

To exclude a direct effect of reserpine infused ICV on colitis, the same dose of reserpine was given into the peritoneum via a MOP at 1 μg/day for 14 days; reserpine did not modify the colitis in mice in the absence of preexisting depression (Supplementary Figure 1). Reserpine and DMI treatments showed the same results when chronic DNBS colitis was reactivated or not (Table 1).

**Effect of Depression on Colonic Barrier Function**

We next evaluated whether colonic barrier function, as assessed by ion transport and conductance, was affected when the colitis was reactivated without any new DSS challenge. No statistically significant changes were found in barrier function in mice that had recovered from chronic DSS colitis previously and subject to depression or subject to depression and treated with DMI. The short-circuit current (Isc) values were 65.1 ± 6.6, 53.9 ± 11.1, and 83.1 ± 11.4 μA/cm², respectively. The conductance, reflecting the paracellular transport, was not significantly changed in RM-DMI treated mice; RM-treated mice compared with nontreated mice. Values were, respectively, 24.5 ± 2.4, 25.9 ± 2.3, and 22.4 ± 3.2 ms/cm².

**Role of the α7nAChR**

Next, we examined whether treatment with selective choline-chloride and methyllycaconitine, respectively, α7nAChR agonist and antagonist, influences the depression-induced reactivation of colitis. Treatment with CC significantly decreased all inflammatory markers in RM. Conversely, MLA did not increase further the inflammatory markers in those mice when reactivated with DSS 2%, except for the macroscopic scores (Supplementary Figure 2). In the absence of reactivation, CC also decreased the macroscopic scores, MPO, IL-6, and IL-1β by 78%, 44%, 34%, and 54%, respectively, in RM (Figure 3). No significant changes were seen when treated with MLA. In contrast, in RM treated with DMI, MLA abolished the beneficial effect of the antidepressant on reactivation by depression. Macroscopic scores and MPO were increased by 3.5- and 4.9-fold, respectively, and IL-6 and IL-1β by 5.9- and 12-fold, respectively.

![Figure 3](image.png)

Figure 3. Influence of nicotinic α7 agents on depressed mice. Influence of nicotinic α7 agents on reserpinized mice (RM) and RM-treated with desmethylimipramine (DMI, 15 mg/kg/day, IP). The specific α7 agonist choline-chloride (CC) decreased significantly all the different inflammatory markers in RM. The specific α7 antagonist methyllycaconitine (MLA) did not modify the different inflammatory markers in RM. The benefit effect of DMI was abolished when mice were treated with MLA. (A) Macroscopic score, (B) myeloperoxidase (MPO) activity, (C) interleukin (IL)-6 cytokine level in colonic tissue, and (D) IL-1β cytokine level in colonic tissue. *<sup>P</sup> < .05, n > 8. The values are shown as means ± SEM.
To determine whether MLA and CC act centrally when injected peripherally, we then administered these agents ICV. Neither MLA or CC modified the reactivation of colitis in mice without depression (Supplementary Figure 3). These results indicate that the α7nAchR is critical for the reactivation of colitis by depression.

To confirm the role of the α7nAchR on DSS colitis, we used α7-deficient mice. However, these knockout mice did not survive a second cycle of DSS treatment (Supplementary Figure 4). Experiments were performed using a single trial of 5 days of DSS 5%, which induced acute colitis. The absence of α7nAchR accelerated the onset of loose stools, weight loss, and rectal bleeding. On day 3 postcolitis induction, 55% and 95% of α7-deficient DSS-treated mice had rectal bleeding and weight loss, respectively, compared with 30% and 70%, respectively, of wild-type DSS-treated mice ($P < .05$) (data not shown). On day 5 postcolitis induction, macroscopic scores, MPO, and CRP levels were 111%, 73%, and 35%, respectively, higher in α7-deficient DSS-treated mice compared with wild-type (Figure 4A and 4B). IL-6 and IL-1β tissue levels were 31% and 96%, respectively, higher in α7-deficient DSS-treated mice (Figure 4C). Histologic scores increased from 1.8 ± 0.4 to 3.7 ± 0.6 in α7-deficient DSS-treated mice compared with wild-type DSS treated, respectively. Pretreatment with nicotine or CC significantly decreased these markers in DSS-treated wild-type mice, but no differences were seen in α7-deficient mice treated with nicotine. Hexamethonium or MLA treatment significantly increased these markers in the wild-type DSS-treated mice, but no differences were seen in α7-deficient mice treated with hexamethonium. Vagotomy had no effect on the colitis in α7-deficient mice (Figure 4). These data confirm the role of this receptor in the expression of colitis.

Effect of Depression on Macrophages

We next assessed the functional role of the macrophage in the reactivation of colitis induced by depressive-like behavior. Reactivation of colitis without any new DSS challenge was associated with a transmural infiltrate of F4/80+ macrophages in non-RM and RM treated or

![Figure 4](image_url)  
**Figure 4.** Influence of selective and nonselective nicotinic α7 agents on wild-type and α7-deficient mice. (A) Myeloperoxidase (MPO) activity, (B) C-reactive-protein (CRP) level, and (C) interleukin (IL)-6 and IL-1β cytokine level in colonic tissue after 5 days of dextran sulfate sodium (DSS)-induced colitis on α7-deficient mice. Selective and nonselective nicotinic α7 agonist and antagonist decreased and increased, respectively, all the different markers on wild-type mice. Nonselective nicotinic α7 agents did not show any effect on α7-deficient mice. *$P < .05$* compared with wild-type mouse, $n > 8$. The values are shown as means ± SEM.

![Figure 5](image_url)  
**Figure 5.** Role of the macrophage in the reactivation of colitis. (A) Accumulation of F4/80+ cells with previously established chronic DSS colitis induced in nondepressed, depressed, and depressed desmethylimipramine (DMI) treated mice. F4/80 immunostaining of colon tissue of nonreserpinized (non-RM), reserpinized (RM), and reserpinized nonreactivated mice treated with DMI. F4/80+ area data are expressed as the percentages of the positive staining areas of the total area. Original magnification, 100×. (B) LPS-stimulated (100 ng/mL) mouse macrophage cultures from non-RM or sham-Obx, RM or Obx, and RM or Obx treated with DMI were incubated with choline-chloride (CC) or interferon-γ (INF). Interleukin (IL)-1β and IL-6 amounts were measured in conditioned media (3 hours) using commercially available enzyme-linked immunosorbent assay kits. *$P < .05$*; the values are shown as mean ± SEM from 4 separate experiments.
not with antidepressant. F4/80+ cells were evident in mucosa, submucosa, and muscle layers in mice with previous chronic colitis (Figure 5A). To determine whether the macrophage sensitivity was modified by depression, we examined the cytokine release from intraperitoneal macrophages (Figure 5B). We stimulated cultured mouse macrophages with LPS (100 ng/mL) and treated them with CC (10⁻⁶ mol/L) or INF-γ (150 IU/mL). Cells from depressed mice released an increased release of IL-6 and IL-1β following LPS stimulation compared with nondepressed mice. However, this was not evident in macrophages issued from TCA-treated, depressed mice. To determine the capacity of these cells from nondepressed mice to produce proinflammatory cytokines, we costimulated cells with INF-γ and achieved a similar level of secretion of IL-6 and IL-1β as was seen in macrophages from depressed mice. Because CC attenuates the effect on depressed mice in vivo, we next examined whether it could decrease the release of cytokine on macrophage isolated from depressed mice. As reported, CC decreased significantly IL-6 and IL-1β (Figure 5).

Discussion

Depression is common in IBD and most likely reflects the morbidity associated with these conditions. The results of this study provide proof of concept that depression per se can reactivate intestinal inflammation in mice in which chronic inflammation had been induced and allowed to become clinically quiescent. This model is reminiscent of the clinical scenario in which depression may occur during the natural history of IBD. Our results show that the induction of depression reactivates gut inflammation in 2 experimental models of colitis by attenuating the α7nAchR-mediated vagal suppression of proinflammatory cytokine release from macrophages. Furthermore, we showed that treatment with TCA attenuates the depression-induced reactivation of gut inflammation. These results have clinical and scientific implications in the management and understanding of chronic intestinal inflammation. A major observation is the finding that TCA inhibited the reactivation of colitis by depression. Critical to this interpretation is the finding that TCA did not influence inflammation in the absence of depression. We are aware of the possibility that the TCA may have direct antiinflammatory effects, but these were not evident in our experiments. DMI had no effect on any parameter of inflammation in DSS-treated mice without depression.

Nicotinic acetylcholine receptors (nAchR) are present along the gastrointestinal tract of animal and human, and there are reports that nicotine interacts with the α7nAchR on macrophages. We hypothesized a possible association among α7nAchR, depression, pathogenesis, and course of intestinal inflammation. Our results are consistent with the notion showing that the tricyclic antidepressant DMI attenuates the inflammatory response in RM by enhancing parasympathetic function. First, the deleterious effect of depression on reactivation of colitis is absent in CC-treated mice. Moreover, MLA therapy did not up-regulate inflammation in depressed mice. Second, the beneficial effect of DMI was absent in

Figure 6. Bulbectomy reactivates DSS-induced colitis. (A) Influence of bulbectomy (obx) on disease activity index (DAI). Day 7 (D7) postsurgery, the DAI started to increase significantly without any new challenge. Desmethylimipramine (DMI; 15 mg/kg/day, IP) treatment decreased significantly the DAI. (B) Effect of Obx on reactivation of colitis with dextran sulfate sodium (DSS) 2%. Macroscopic score, MPO activity, and cytokine level in colonic tissue after reactivation were increased in Obx mice. Treatment with DMI decreased significantly all the markers in nonreactivated group. **P < .05, n > 8, vs sham nonreactivated. *P < .05, n > 8, vs obx nonreactivated. The values are shown as means ± SEM.

Effect of Bilateral Olfactory Bulbectomy on Quiescent Colitis in Mice and Cultured Macrophages

To determine the specificity of our findings with reserpine, we used another model of depression that is well established in the literature: the model of bilateral olfactory bulbectomy (Obx). On day 7 postsurgery, 50% Obx mice had loose stools compared with 12.5% of sham-operated, DSS-treated mice (Figure 6A). Obx mice exhibited a more severe reactivation of the colitis compared with sham mice treated or not with 2% DSS. The effect of Obx was significantly decreased when mice were treated with DMI (Figure 6B). The assessment of the functional role of the macrophage in the reactivation of colitis induced by Obx revealed a profile similar to that found in the reserpine model (Figure 6B).
trophils express nicotinic receptors, it is yet unclear whether these effects are mediated by the α7nAChR. In contrast to previously published data on stress reactivation, MLA-treated mice in the absence of reactivation of colitis, and treatment with CC did not further decrease the inflammatory markers. Whereas it is known that nicotine has antidepressive effects that could affect vagal activity and influence the effects of MLA and CC peripherally, we found no evidence of a central effect of these compounds on colitis.

We confirmed a major role of the α7nAChR in depression-induced reactivation because α7-deficient mice showed an increased severity colitis induced by DSS and neither the receptor agonist nor the antagonist modulated the degree of inflammation in α7-deficient mice. In addition, vagotomy did not influence the severity of colitis in α7-deficient mice. The importance of α7 receptor in our model is similar to that found in sepsis and pancreatitis. Macrophages are an important component of the inflammatory response in murine models and in human IBD and are responsible for the production of proinflammatory cytokines. Several groups have identified the α7nAChR on human and murine macrophages. In vitro examination of intraperitoneal macrophages from depressed mice revealed an increased release of proinflammatory cytokines following LPS stimulation and that was not evident in CC-treated macrophages or cells issued from TCA-treated, depressed mice. This is further supported by demonstrations in vitro that α7nAChR are involved in the selective down-regulation of LPS-induced release of tumor necrosis factor-α, IL-6, and IL-1β in cultured macrophages. We conclude that the effect of depression is accompanied by macrophage impairment associated with an increase of sensitivity of these cells to LPS.

It is likely that other factors contributed to the deleterious effect of depression on colitis in our study. Parasympathetic impairment results in a dominant sympathetic drive, and it is known that this can enhance or decrease colonic inflammation. In addition, a decrease of the vagal activity alters lymphocyte trafficking and mast cell numbers in the gut and influences gut physiology, and these factors could contribute to the changes in severity of the reactivation of colitis seen in our study. In addition to macrophages, nicotine alters various neutrophil functions such as chemotaxis and superoxide anion production. However, although neutrophils express nicotinic receptors, it is yet unclear whether these effects are mediated by the α7nAChR. In contrast to previously published data on stress reactivation of colitis, the reactivation seen here is not due to changes in intestinal barrier function.

In conclusion, the present study provides proof of concept that depression reactivates quiescent inflammation in the bowel and elucidates the underlying pharmacologic mechanism. These findings have important clinical implications because they clarify uncertainty regarding the link between depression and IBD. Our results emphasize a causal linkage between depression and relapse of IBD and encourage close monitoring of depressive behavior in IBD patients. The results demonstrate a role for antidepressants in stabilizing the natural history of the disease and provide a basis for considering trials of highly selective nAChR agonists to prevent relapses in patients with IBD and depression.

**Supplementary Data**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at [www.gastrojournal.org](http://www.gastrojournal.org) and at doi: 10.1053/j.gastro.2009.02.069.

**References**


Supplementary Materials and Methods

Chronic ICV and peritoneal infusion. The therapeutic use of reserpine for hypertension resulted in realization that it could induce severe depression. Reserpine acts by depleting biogenic amines. In rodents, acute administration of a large dose of reserpine IP depleted concentrations of noradrenaline, adrenaline, dopamine, and serotonin in the brain for more than a week but depression persisted for only 72h. Therefore in our study we induced a sustained depression by administering a low dose of reserpine by ICV injection for 14 days. Forty-eight days after the first challenge with DSS or DNBS, MOP (Alzet, Cupertino, CA) were filled with vehicle (acetic acid 0.2%), reserpine solution (1 μg/day for 14 days). Previously, we showed that a treatment with anti-depressant two days after the surgery could re-increase the level of 5-HT3 into the brain. The MOP were connected by a 1.5–2 cm length of PE-60 tubing to a 3-mm-long cannula (Plastics One, Roanoke, VA) and sterilized. After anesthesia with ketamine (150 mg/kg, IP) (Bimeda-MTC, Cambridge, Ontario, Canada) and xylazine (10 mg/kg, IP) (Bayer HealthCare, Toronto, Ontario, Canada) the scalp was shaved, an incision was made along the midline and the skull was scraped clean of periosteum. Hemostats were used to make a pocket under the skin between the shoulder blades. The MOP was placed in this pocket, the cannula was inserted through the drilled hole into the lateral ventricle, and the cannula pedestal was affixed to the skull with cyanoacrylate glue (Loctite, Mississauga, Ontario, Canada) and xylazine (10 mg/kg, IP) (Bayer HealthCare, Toronto, Ontario, Canada) to reduce the post-operative pain. Sham animals were treated identically, with the exception that the olfactory bulbs were not removed. The mice were allowed to recover for 9 days and were handled everyday to decrease hyperirritability and aggression, before commencement of any experimental procedure.

Experimental depression-related protocol. After establishment of chronic colitis, tail-test was performed two days before surgery (day 46). Placement of the MOP or obx were performed on day 48. Mice were tested for depression-like behaviour on day 8 post-surgery. As previously described, DMI (Sigma–Aldrich, Mississauga, Ontario, Canada) was administrated i.p. at the dose of 15 mg/kg for 12 days starting 2 days post-surgery. In separate experiments, CC (Sigma–Aldrich) (20 μg/ml) were added to the drinking water on day 4 post-surgery and for 5 days after the re-induction of colitis according the group tested. In separate experiments, MLA (Sigma–Aldrich) (5 mg/kg) was administered by s.c. injection twice a day for 5 days post-DSS. In control experiment, the MOP was loaded with MLA or CC. Reactivation of colitis with 2% DSS commenced on the 9th day post-surgery and continued for 5 days and exposure to DNBS (3 mg in 30% ethanol) commenced on the 11th day post surgery for 3 days.

Assessment of the severity of colitis—DAI. DAI scores have historically correlated well with the pathological 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: 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; and 4, gross blood (Hemmoccult II, Beckman Coulter, Fullerton, CA). DAI was scored from day 0 to day 5 during DSS treatment.

Colonic histology and MPO activity. Formalin-fixed colon segments coming from the splenic flexure were paraffin-embedded and 3-μm sections were stained with hematoxylin-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 infiltrate. MPO activity was determined following an established protocol. Briefly, MPO activity, used as a marker of granulocyte infiltration, was extracted and the activity was measured using a modified version of the method described by Bradley. Tissue samples were homogenized (50 mg/ml) in ice-cold 50mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethyl ammonium bromide (Sigma–Aldrich). 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 and hydrogen peroxide (Sigma–Aldrich). The absorbance of the colorimetric reaction was measured by a spectrophotometer at 450 nm. MPO is expressed in units.
per milligram of wet tissue, 1 unit being the quantity of enzyme able to convert 1 mM of hydrogen peroxide to water in 1 minute at room temperature.  

**Macrophages staining.** Serial sections were deparaffinized in CitriSolv (Fisher Scientific, Illkirch, France) and rehydrated through a graded series of ethyl alcohol and PBS. Endogenous peroxide was blocked by an incubation in peroxidase blocking reagent (DakoCytomation, Carpinteria, CA) for 15 min. After being washed, sections were predigested with proteinase K solution (DakoCytomation) for 15 min at room temperature or were subjected to antigen retrieval in citrate buffer (pH 6.0) after being heated in a microwave. After being washed and blocked of nonspecific binding with 1.0% BSA in PBS, sections were incubated with monoclonal rat anti-mouse antibody F4/80 (Serotec, Raleigh, NC, 1:100, 18 h at 4°C). After being washed, sections stained for F4/80 immunostaining were incubated with biotinylated goat anti-rat IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, 1:200) for 1 h followed by horseradish peroxidase-conjugated streptavidin (DakoCytomation, 1:300) for 30 min. Sections were developed with 3,3'-diaminobenzidine solution as a chromogen. They were counterstained with Meyer’s hematoxylin (DakoCytomation), dehydrated, cleared, and mounted. Negative controls were prepared by omission of the primary antibodies.

### References
Supplementary Figure 2. Influence of nicotinic α-7 agents on reactivation of DSS-induced colitis in depressed mice. Influence of nicotinic α-7 agents on reactivation of colitis with DSS on RM. The specific α-7 agonist, CC, decreased significantly all the different inflammatory markers. The specific α-7 antagonist, MLA, did not modify the different inflammatory markers except for the macroscopic score. (A) Macroscopic score, (B) MPO activity, (C) IL-6 cytokine level in colonic tissue, and (D) IL-1β cytokine level in colonic tissue. *P < .05, n > 8. The values are shown as means ± SEM.

Supplementary Figure 3. Chronic ICV infusion of nicotinic α-7 agents did not modify DSS-induced colitis. Effect of ICV infusion of nicotinic α-7 agents on reactivation of colitis with DSS. Inflammatory markers were significantly modified by neither CC nor MLA. (A) Macroscopic score, (B) MPO activity, (C) IL-6 level in colonic tissue, and (D) IL-1β level in colonic tissue. The values are shown as means ± SEM, n > 8.
Supplementary Figure 4. Survival rate of α7-deficient mice after two cycles of DSS. Comparison between wild-type (+/+) and α7-deficient mice (−/−).