

Antidepressants Attenuate Increased Susceptibility to Colitis in a Murine Model of Depression

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Background & Aims: Psychiatric factors may determine gastrointestinal health outcomes. Here, we used a model of depression based on neonatal maternal separation (MS) to identify alterations in gut physiology and to assess its association with increased sensitivity to experimental colitis in adulthood. We also examined whether antidepressant therapy attenuates the increased susceptibility to colitis. **Methods:** C57BL/6 mouse pups were separated from mothers for 3 hours per day at 1–21 days of age. Maternally unseparated (US) litters served as controls. At 8 weeks of age mice were examined for changes in behavior, intestinal permeability, and sensitivity to colitis. Separate sets of MS and US mice were given either saline or the antidepressant desipramine 15 mg/kg once daily at 23–36 days of age. Testing of mice occurred at 8 weeks of age. **Results:** Adult MS mice showed evidence of depressive-like behavior and enhanced intestinal permeability but showed no evidence of spontaneous inflammation. A more severe colitis was seen in MS compared with US mice. Antidepressant therapy improved parameters of depressive-like behavior and reduced the vulnerability to dextran sulphate sodium colitis in MS mice but had no effect on colitis in US mice. **Conclusions:** MS may lead to depression and increased responsiveness to stress, to impaired intestinal barrier function, and to enhanced vulnerability to colitis in adulthood. This vulnerability is reversed by antidepressant therapy. Depression increases vulnerability to intestinal inflammation. We speculate that pre-existing depression may facilitate the expression of inflammatory bowel diseases.

Although ulcerative colitis initially was considered to represent a psychosomatic disease,¹ the role of behavioral factors in inflammatory bowel disease (IBD) is controversial.² With increased understanding of the role of the immune system in the pathogenesis of IBD,³ less attention has been paid to the impact of behavioral factors on the natural history of these diseases. Some studies have shown that there is a higher than expected incidence of depression in both ulcerative colitis (UC)⁴ and Crohn's disease (CD),⁵ and in other studies depres-

sion correlated well with disease activity, suggesting that it might be secondary to the disability imposed by IBD.⁶ However, in another study depression was unrelated to disease activity,⁷ and in other studies it actually predated the onset of CD⁸ and UC.⁴ It is therefore unclear whether depression is merely an unrelated epiphenomenon that occurs as a result of the disease and the disability it imposes, or whether the presence of depression plays a role in facilitating the expression of IBD.

Although major depressive disorder has long been associated with central neural changes such as cognitive impairment⁹ and hippocampal atrophy,¹⁰ it also has been linked with an increased risk for hypertension,¹¹ type II diabetes,¹² peptic ulcer disease,¹³ immune dysfunction,¹⁴ and cardiovascular disease.^{15,16} The increased risk for ischemic heart disease in depressed patients has been linked with increases in the acute phase reactant C-reactive protein.^{16–18} Indeed, depression and anger per se may result in increased C-reactive protein levels,¹⁷ thus creating a potential link between behavior and risk for inflammatory-based disorders.

Childhood exposure to emotional trauma, such as maternal loss, is recognized as a risk factor for the development of psychiatric disorders, including depression in adulthood.¹⁹ During the postnatal period, the infant depends on the mother not only for nursing and protection but also for normal brain development.²⁰ Rodent models have shown that neonatal maternal separation (MS) leads to depressive-like behavior that may be treated with antidepressants in adult life.²¹ We exploited this model to test the hypothesis that exposure to neo-

Abbreviations used in this paper: ⁵¹Cr-EDTA, 51-chromium-ethylenediaminetetraacetic acid; CRF, corticotropin-releasing factor; DMI, desipramine; DNB, dinitrobenzenesulfonic acid; DSS, dextran sulphate sodium; IL, interleukin; MPO, myeloperoxidase; MS, maternal separation; PND, postnatal day; SAP, serum amyloid P-component; US, unseparated.

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natal psychologic stress that induces depressive-like symptoms leads to altered intestinal physiology and an enhanced vulnerability to intestinal inflammation in adulthood. To investigate a causal link between the behavioral changes and intestinal events, we also examined whether antidepressant therapy of MS mice attenuated the susceptibility to inflammatory stimuli later in life.

Materials and Methods

Animals

Specific pathogen-free, pregnant C57BL/6 female mice (whose conception date was known) were obtained from Taconic Farms (Germantown, NY) on gestational days 15–16. Dams were housed individually in cages containing bedding material on a 12-hour light-dark cycle (lights on at 8:00 AM) and provided with food and water *ad libitum*. All procedures were approved by the Animal Rights Ethics Board at McMaster University.

Maternal Separation

The MS protocol used in the present study was a slight modification of one previously published.²¹ Dams and their litters were assigned at random to the control (unseparated) group or the MS group. MS pups were removed from their home cages and dams at postnatal day (PND) 1 to PND 21 for 180 minutes daily by placing them as a litter in a new isolation cage. The isolation cages were lined with chip bedding and kept at $37^{\circ}\text{C} \pm 5^{\circ}\text{C}$ by using a heating pad placed under the cages. Dams of the MS group also were removed from the home cages and transferred to separate holding cages during the MS procedure. The technician's gloved hands were rubbed in the bedding of each litter before handling of pups to prevent rejection by dams on reunion. Unseparated (US) litters were left undisturbed, except for routine cage care by the technician. At weaning (PND 23), male offspring were identified and served as subjects in the study. Subjects were weighed at PND 60.

Antidepressant Treatment After MS

Treatment of animals with the antidepressant desipramine (DMI) after MS was according to a slight modification of a previously published regimen.²¹ DMI treatment began on PND 23. Half of the animals in both the MS and US groups were given intraperitoneal injections of 15 mg/kg DMI once daily between 10:00 AM and 12:00 PM, whereas control animals from both the MS and US groups received equivalent volumes of saline intraperitoneally. DMI or saline treatment continued until PND 36. After completion of the DMI treatment, animals were left undisturbed except for routine cage cleaning until behavior (tail suspension) testing or colitis induction was begun on PND 60.

Antidepressant Treatment in Adult US Mice

To examine any direct effect of desipramine on colitis, DMI was administered once daily by intraperitoneal injections (15 mg/kg) for 1 week before and during induction of colitis by dextran sulphate sodium (DSS) in drinking water.

Tail Suspension Test

The tail suspension test, conducted as previously described, is a test of depressive-like behavior in rodents.²² Mice were fastened securely by the distal end of the tail to a flat metallic surface and suspended in a visually isolated area ($40 \times 40 \times 40$ cm white Plexiglas box). The presence or absence of immobility, defined as the absence of limb movement, was sampled every 5 seconds over a 6-minute test session by a highly trained observer who was blinded to neonatal manipulation.

Assessment of Behavioral Responses to Stress

Adult mice at PND 60 were tested for evidence of increased behavioral responsiveness to stress, consistent with depressive-like symptoms as suggested by previous studies.²¹ The open field test and the novel object tests are used to assess behavioral reactivity to stress by exposing rodents to novel environments and novel objects, respectively, and combinations of both tests also may be used.²³ The behavior tests used in the present study were slightly modified from those published previously.²⁴ Two weeks before the start of the experiment (at PND 46), mice were separated from littermates and were housed individually. Mice were handled briefly by the experimenter every day for 5 days before the start of the experiment. Testing was conducted between 6 PM and 12 AM in a dimly lit room.

Each mouse was placed gently in the center of an open field (a Plexiglas cube [$450 \times 450 \times 450$ cm] custom built at McMaster University) and allowed to explore the novel environment for 10 minutes. At 10 minutes, a novel object (a white Styrofoam cup) was introduced into the center of the field and mice were allowed to respond to the novel object for a further 10 minutes. All sessions were filmed using a Sony video camera (Toronto, Canada). At the end of each session the open field was cleaned with paper towels that were moistened with an ammonium glass cleaner (Windex, SC Johnson & Son, Inc., Brantford, Ontario, Canada) to remove urinary trails. During each session, mice from both the MS and US groups were tested simultaneously by placing 2 open fields adjacent to each other under the camera's optical field. Locomotor activity of mice throughout the 20-minute trial was measured from the video records using the EthoVision 2.3 tracking software system (Noldus Information Technology, Leesburg, VA). The distance traveled per movement bout (the mean length of a locomotor bout) was chosen as the index of stress reactivity to the novel environment because it combines both the amount and rate of activity. The time-course of the amount and duration of locomotion across the session was taken as a

reflection of the rate of habituation to the stressful novel environment.

Assessment of Feeding Patterns

Feeding patterns were assessed over 24 hours in MS and US adult mice on PND 60. Two weeks before the start of the experiment, mice were separated from littermates and were housed individually. The experimental feeding apparatus consisted of food pellets fastened onto feeding trays (diameter, 3 cm) positioned 5 cm above the bottom of the cage. Mice were allowed to habituate to the experimental feeding trays for 1 week before the start of experiments. During the experiment, day/night cycles were adjusted with lights turning on at 7:30 AM and turning off at 7:30 PM. At the start of the experiment, feeding trays were connected to computer-monitored force transducers placed above the cage. Weights of the feeding trays with food pellets were recorded continuously throughout the experiment by custom-written computer software, Acquire 5.1 and GraphView 5.1. The beginning of each feeding bout was marked by an abrupt increase in weight (as the mouse leaned on the feeding tray to gain access to the food pellets), and the end of the bout was characterized by an abrupt return to near baseline weight (as the mouse released the feeding tray). The difference in weight between before and after a feeding bout corresponded to the amount of food consumed during that bout. A feeding bout was defined as an episode of food consumption lasting more than 20 seconds; 2 bouts were considered independent from each other if the interval of inactivity was longer than 5 minutes, as defined previously.²⁵

Intestinal Permeability Measurement

Adult mice underwent intestinal permeability testing on PND 60. Isolation and preparation of intestinal loops were according to a protocol by Bercik et al²⁶ that was adapted to mice for the assessment of intestinal permeability using recovery of radioactivity in venous outflow after intraluminal perfusion of jejunal loops with 51-chromium-ethylene-diaminetetraacetic acid (⁵¹Cr-EDTA). The luminal perfusate containing ⁵¹Cr-EDTA was a slight modification of the solution used previously²⁷ in clinical studies of intestinal permeability.

Briefly, mice were anesthetized with intraperitoneal ketamine/xylazine. After a midline incision was made, a 2.5- to 3.5-cm segment or loop of the jejunum was selected and the terminal branch of the superior mesenteric artery was cannulated with a polyethylene catheter. Tissue oxygenation was maintained by perfusion of the arterial branch with Hemolink, a hemoglobin-based oxygen carrier, in lactated Ringer's solution (Hemosol Inc., Mississauga, ON) equilibrated with O₂ (100%) using a peristaltic pump (Ismatec SA, Zurich, Switzerland). Nutrition for the loop was provided by the addition of 0.1% (wt/vol) glucose and 0.6 mmol/L glutamine to the arterial perfusate. Normal mouse serum 10% (wt/vol) also was added to the arterial perfusate to prevent methylation of hemoglobin and to provide additional nutrition. Both oral and aboral ends of the jejunal segment were cannulated using

polyethylene cannulas and fastened with double ligatures. The jejunal loop was dissected from its mesentery and transferred to an organ chamber containing phosphate-buffered saline (PBS) at 37°C and perfused intraluminally in an aboral direction with PBS at 5 mL/h using a syringe infusion pump (Harvard, Boston, MA).

After isolation of the intestinal loop, all preparations were allowed to equilibrate for 10 minutes before venous outflow collection. Venous outflow was collected continuously for the remainder of the experiment in 3-minute fractions. After equilibration, perfusion of PBS was stopped and the lumen was perfused with the ⁵¹Cr-EDTA solution for 15 minutes at 5 mL/h. The ⁵¹Cr-EDTA solution, adjusted to 300 mOsm, contained ⁵¹Cr-EDTA (0.6 μCi/mL) (Perkin Elmer, Boston, MA), mannitol (3 mg/mL) (Sigma-Aldrich Corporation, Oakville, Ontario, Canada), and NaCl (0.4% wt/vol) (Sigma-Aldrich Corporation) in distilled H₂O. At 15 minutes, intraluminal perfusion of the ⁵¹Cr-EDTA solution was stopped and the lumen was perfused with PBS at 5 mL/h for a wash period of 30 minutes. After the end of each experiment, the length of the jejunal loop was measured and 2 full-thickness tissue samples were excised from proximal and distal regions of the loop, fixed in formalin, stained with H&E, and examined for tissue damage by light microscopy.

The γ radiation from ⁵¹Cr-EDTA in the venous outflow fractions was measured by counting a 150-μL aliquot of each fraction in a well-type γ-counter (1282 Compugamma, LKB WALLAC; Fisher Scientific, Toronto, ON) for 1 minute. Counts were compared with a ⁵¹Cr-EDTA standard curve prepared previously from the luminal perfusate. The recovery of radioactivity in each venous outflow fraction was calculated as a proportion of that found in an identical volume of the luminal perfusate, corrected for length of the jejunal loop. The total recovery of radioactivity was determined as the sum of the radioactivity recovery of individual fractions.

Assessment of Spontaneous Inflammation

MS and US mice were killed on PND 60 for assessment of basal-state immunity. Whole blood was collected by cardiac puncture in mice while under anesthesia before death for a serum amyloid P-component (SAP) level measurement. Samples of the distal colon were prepared for histology by fixation in 10% formalin followed by H&E staining. Additional samples of the distal colon were snap-frozen in liquid nitrogen for assessment of myeloperoxidase (MPO) activity and inflammatory cytokine levels.

Histologic Assessment of Colonic Tissue

Microscopic scoring was based on a scheme that accounted for the loss of mucosal architecture and infiltration by inflammatory cells.²⁸ Microscopic scoring was conducted by 2 experimenters in a blinded fashion.

MPO Assay

Acute inflammation was assessed by MPO activity in intestinal tissue. The assay was performed on frozen samples as

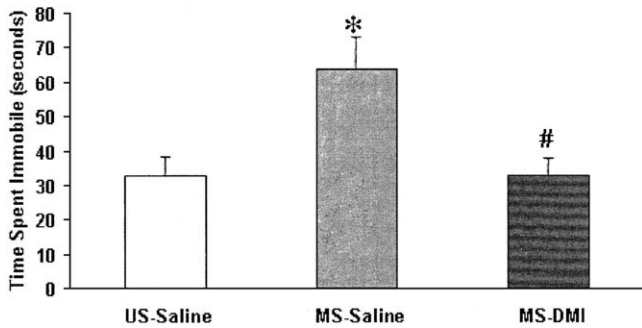


Figure 1. Immobility in MS adult mice before and after DMI treatment. Total time spent immobile is represented in seconds and was measured in mice on PND 60. MS mice were given DMI (MS-DMI; $n = 8$) or a saline vehicle (MS-saline; $n = 4$) on PND 23–36. US mice were given injections of saline vehicle (US-saline; $n = 4$). Data are means \pm SEM. * $P < .05$ compared with US-saline mice; # $P < .05$ compared with MS-saline mice.

described previously.²⁹ MPO activity is reported in units per mg of wet tissue, where 1 unit of MPO is defined as the quantity of enzyme able to convert 1 μ mol of hydrogen peroxide to water in 1 minute at room temperature.

Tissue Cytokine Assay

Levels of interleukin (IL)-1 β were assessed in intestinal tissue. Total protein was extracted from frozen tissue according to a protocol previously described.³⁰ Total protein levels were measured using a commercially available variant of the Lowry assay (Bio-Rad Laboratories, Hercules, CA). IL-1 β levels in the protein extracts were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). IL-1 β levels are reported as pg of IL-1 β per mg of total protein.

SAP Enzyme-Linked Immunosorbent Assay

SAP is considered the prototype acute-phase reactant in mice.³¹ Serum was extracted from whole-blood samples by centrifugation. SAP levels were measured using a sandwich enzyme-linked immunosorbent assay technique according to Taktak and Stenning.³¹ SAP levels are reported as μ g of SAP per mL of serum.

DSS-Induced Colitis

Acute colitis was induced in adult mice by 6% DSS in drinking water for 5 days (PNDs 60–65). At the end of DSS feeding, blood was obtained for SAP levels. Clinical disease scores graded the presence of rectal prolapse, rectal bleeding, and weight loss as described previously.²⁸ Samples of the distal colon were obtained for histology, assessment of MPO activity, and IL-1 β levels.

Dinitrobenzenesulfonic Acid-Induced Colitis

Colitis was induced by intracolonic administration of 6 mg dinitrobenzenesulfonic acid (DNB) in a 50% ethanol solution as previously described.³² The mortality level at day 3 post-DNB administration was used as a measure of vulnerability to colitis. Mice surviving beyond day 3 post-DNB

administration were not assessed for colitis severity owing to inadequate numbers for statistical testing.

Statistical Analysis

Data are means \pm SEM. Measures of locomotion were analyzed using a 2-way analysis of variance with repeated measures²⁴ where 1 factor was between-subjects (neonatal manipulation with 2 levels: MS vs US), and the other factor was a repeated-measures factor also with 2 levels (time interval, 0–10 min vs 10–20 min). The tail suspension test was analyzed using a 1-way analysis of variance with a Bonferroni posttest. Statistical testing for the remaining experiments was performed using a t test. In all cases a P value of less than .05 was considered significant.

Results

Tail Suspension Test Before and After Antidepressant Treatment

DMI treatment attenuated depressive-like behavior in adult MS animals. As shown in Figure 1, maternally separated animals spent more time immobile (63.75 ± 9.44 s) as compared with US animals (32.75 ± 5.33 s; $P < .05$). Treatment with desipramine significantly attenuated immobility in maternally separated animals (32.88 ± 4.92 s; $P < .05$). Significant differences in immobility were not seen between unseparated animals and maternally separated animals treated with DMI.

Behavioral Responses to Stress

As shown in Figure 2, MS mice traveled less distance per movement bout during the 20-minute exposure to the open field as compared with US mice (neonatal manipulation effect, $F(1,34) = 5.08$; $P = .031$). After the introduction of a novel object after 10

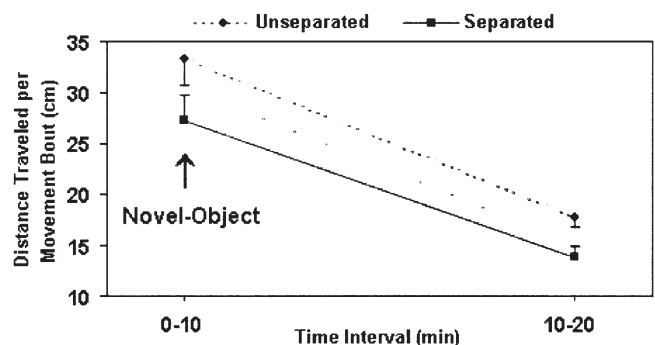


Figure 2. Pattern of novelty-induced locomotor activity in adult MS and US mice. The distance traveled by mice per movement bout (mean length of locomotor bouts) is shown; arrow indicates that a novel object was introduced into the open field after the 10-minute mark. Data are means \pm SEM ($n = 18$ /group). ●, Unseparated; ■, separated.

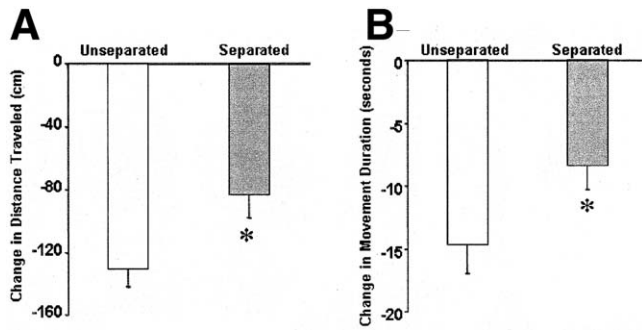


Figure 3. Habituation of novelty-induced locomotor activity in adult MS and US mice. Change from the 0- to 10-minute interval to the 10- to 20-minute interval in (A) distance traveled and (B) locomotor duration. Data are means ± SEM (n = 18/group). *P < .05 compared with unseparated mice.

minutes of exposure to the open field, the mean length of locomotor bouts was shorter compared with the initial 10-minute interval in both MS and US mice (time effect, $F(1,34) = 77.09$; $P = .000$); this decrease in distance traveled per bout was similar for both MS and US mice as confirmed by the absence of an interaction effect between neonatal manipulation and time ($F(1,34) = 0.36$; $P = .553$).

Before the introduction of the novel object, the distance traveled and the duration of locomotion decreased as a function of time during the 10-minute interval in both MS and US mice, reflecting similar habituation for both sets of mice (data not shown). However, MS and US mice responded differently to the introduction of the novel object. As shown in Figure 3A, the decrease in total distance traveled between the time intervals of 0–10 minutes and 10–20 minutes after introduction of the novel object was significantly greater in US mice (-130.94 ± 10.95 cm in US compared with -83.38 ± 14.62 cm in MS mice; $P < .05$). Similarly, as shown in Figure 3B, the change in duration of locomotion was

significantly greater in US compared with MS mice (-14.69 ± 2.26 s in US compared with -8.46 ± 1.77 s in MS mice; $P < .05$). The differences in habituation of novelty-induced locomotor activity between MS and US animals were not a result of incongruous exploration of the novel object. Parameters of contact with the novel object, including latency to first contact with the novel object, frequency of contacts, and duration of contacts were similar in MS and US mice (data not shown).

Body Weights and Feeding Patterns

At 60 days of age, MS mice were significantly heavier than US mice (30.86 ± 0.43 g vs 29.14 ± 0.47 g), respectively ($P < .01$), but this was not owing to altered feeding patterns or to alterations in the amount of food consumed. The total amount of food consumed was similar for both sets of mice. The MS mice consumed 3.10 ± 0.19 g over 24 hours and US animals consumed 3.04 ± 0.19 g ($P > .05$). Feeding patterns were similar between the 2 groups. The total number of feeding periods (23.44 ± 1.56 feeding bouts/24 h in MS mice compared with 23.88 ± 2.67 in controls; $P > .05$) was similar for both sets of mice. The amount consumed per feeding period also was similar between MS and US mice (135.94 ± 10.55 vs 134.32 ± 11.80 mg, respectively; $P > .05$).

Intestinal Permeability

MS mice displayed greater jejunal permeability to $^{51}\text{Cr-EDTA}$ than US mice in adulthood. As shown in Figure 4A, after intraluminal perfusion with $^{51}\text{Cr-EDTA}$, jejunal loops isolated from MS and US animals showed similar patterns of γ radiation recovery in the venous outflow. However, as shown in Figure 4B, the total recovery of $^{51}\text{Cr-EDTA}$ in venous outflow was significantly greater in MS mice compared with US

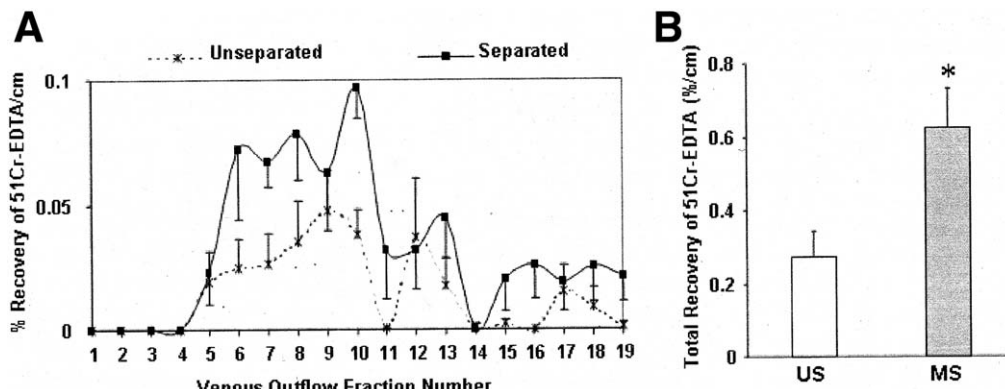


Figure 4. Jejunal permeability to $^{51}\text{Cr-EDTA}$ in adult MS and US mice. Fractions 1–4: luminal PBS; fractions 5–9: luminal $^{51}\text{Cr-EDTA}$; fractions 10–19: luminal PBS wash. (A) Recovery of γ radiation in each venous outflow fraction. *, Unseparated; ■, separated. (B) Total recovery of γ radiation for the duration of the experiment. Data are means ± SEM (n = 5/group). *P < .05 compared with US mice.

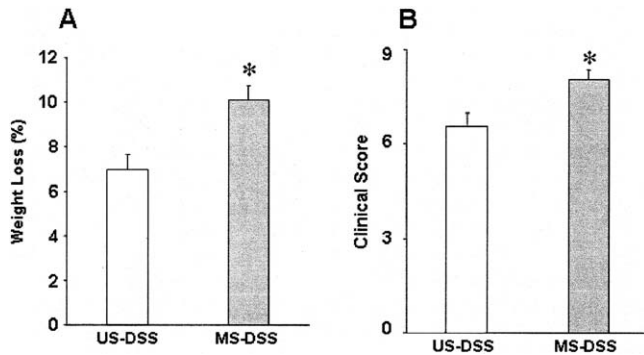


Figure 5. Colitis severity in MS-DSS and US-DSS adult mice after DSS colitis induction. (A) The weight lost after DSS administration is presented as a percentage of the weight at PND 60. (B) Clinical disease score. Data are means \pm SEM ($n = 16$ /group). * $P < .01$ compared with US-DSS mice.

($0.62\%/cm \pm 0.11\%/cm$ vs $0.27\%/cm \pm 0.07\%/cm$ in MS and US mice, respectively; $P < .05$). Histologic sections revealed that the tissue from all mice retained its structural integrity at the end of the experiment (data not shown).

Spontaneous Inflammation in Maternally Separated and Unseparated Mice

Maternally separated mice showed no evidence of spontaneous inflammation in adult life as compared with unseparated mice. There was no evidence of microscopic damage in either MS or US mice and levels of local and systemic markers of inflammation were similar in MS and US mice. IL-1 β was undetectable in colonic tissue from MS and US mice and colonic MPO activity was similar in MS (0.12 ± 0.03 U/mg) and US mice (0.16 ± 0.03 U/mg) ($P > .05$). Similarly, SAP levels were similar in MS (2.96 ± 0.44 μ g/mL) and US mice (3.89 ± 0.60 μ g/mL) ($P > .05$).

DSS Colitis in Maternally Separated and Unseparated Adult Mice

After 6% DSS administration for 5 days, adult MS animals showed a more severe colitis than US animals. As shown in Figure 5A, DSS induced significantly greater weight loss in MS compared with US mice ($10.09\% \pm 0.65\%$ vs $6.96\% \pm 0.68\%$; $P < .01$). Moreover, MS mice showed greater clinical disease scores than US animals (8.06 ± 0.28 vs 6.56 ± 0.42 ; $P < .01$; Figure 5B), greater microscopic damage (2.09 ± 0.22 vs 1.22 ± 0.14 ; $P < .01$; Figure 6A), and a greater infiltration by inflammatory cells in MS-DSS as compared with US-DSS mice (Figure 6B) after induction of colitis.

As shown in Figure 7A, MS mice showed significantly greater MPO activity in colonic tissue compared with US mice (5.29 ± 0.85 U/mg vs 2.63 ± 0.37 U/mg; $P <$

$.01$). Moreover, as shown in Figure 7B, higher IL-1 β levels were detected in colonic tissue from MS mice (40.32 ± 6.00 pg/mg vs 18.06 ± 3.03 pg/mg in MS and US mice, respectively; $P < .01$). As shown in Figure 7C, significantly greater levels of SAP were detected in MS mice compared with controls (931.04 ± 100.51 μ g/mL vs 502.53 ± 77.45 μ g/mL; $P < .01$) after DSS administration.

Colitis Induction After Antidepressant Treatment

DMI-treated MS mice showed a less-severe DSS colitis compared with saline-treated MS mice. As shown in Figure 8, DMI-treated mice had significantly less microscopic damage after DSS colitis (1.4 ± 0.16 and 2.17 ± 0.31 in DMI- vs saline-treated mice, respectively; $P < .05$; Figure 8B) and showed a trend toward less macroscopic damage (3.0 ± 0.29 vs 3.94 ± 0.44 ; $P = .07$; Figure 8A). Furthermore, DMI treatment was accompanied by an attenuation of colonic MPO activity after DSS (0.60 ± 0.10 U/mg vs 3.04 ± 0.95 U/mg in DMI- and saline-treated mice, respectively; $P < .05$; Figure 8C).

DMI treatment of adult mice that had not been subjected to MS did not affect DSS colitis severity. DMI-treated mice had a mean macroscopic score of 1.8 ± 0.8 vs 2.0 ± 0.7 in placebo-treated mice ($P > .05$). Histologic scores were 2.0 ± 0.7 in DMI- and $1.6 (\pm 0.4)$ in placebo-treated mice ($P > .05$).

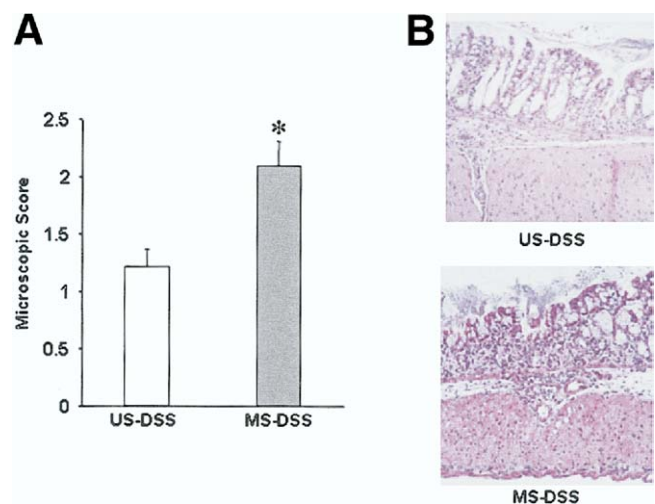


Figure 6. Microscopic damage in MS and US adult mice after DSS colitis induction. (A) Microscopic damage. Data are means \pm SEM ($n = 16$ mice/group). * $P < .01$ compared with US-DSS. (B) Representative histologic sections (200 \times) from both groups.

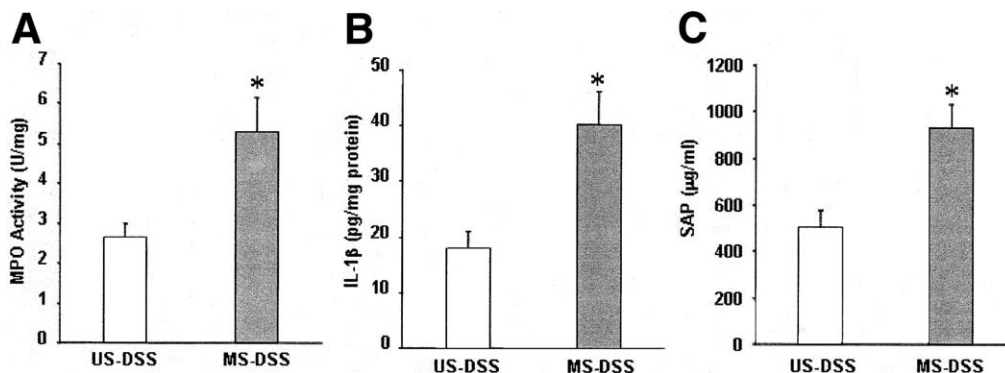


Figure 7. Inflammation in MS and US adult mice after DSS colitis induction. (A) MPO activity in colonic tissue homogenates (n = 16/group). (B) IL-1 β levels in colonic tissue (n = 12/group). (C) SAP levels (n = 16/group). Data are means \pm SEM. *P < .01 compared with US-DSS mice.

DNB Colitis in Maternally Separated and Unseparated Adult Mice

Adult MS mice showed greater vulnerability to DNB-induced colitis as compared with US mice. A 60% (n = 5) mortality rate was observed in the MS group at 3 days after intracolonic administration of 6 mg of DNB in 50% ethanol whereas no mortality was observed in the US group (n = 5). However, tissue was not collected from dead mice for histologic evaluation owing to the presence of autolysis. Mice surviving beyond day 3 after DNB administration were not assessed for colitis severity owing to inadequate numbers for statistical testing. Further experiments were not conducted using the DNB model because death is not a permissible end point at this institution.

Discussion

There is emerging literature linking depression and inflammation; inflammatory cytokines including tumor necrosis factor- α and interleukins 1 and 6 produce behavioral changes similar to those found in depres-

sion.^{33–35} Conversely, depression may be associated with increased production of inflammatory cytokines.³⁴ These findings have prompted suggestions that depression may predispose to inflammatory conditions and there is some evidence to support this with respect to ischemic heart disease.^{16,17} However, little attention has been paid to the role of depression in predisposition to inflammatory conditions of the gastrointestinal tract. In this study, we used MS, a preparation that models behavioral depression to show an increased susceptibility of mice to experimental colitis, and showed that this vulnerability could be reduced by antidepressant therapy.

MS in childhood has been associated with the development of major depression in adulthood.¹⁹ MS also has been used to produce animal models of depression in several species including rodents^{20,21} and primates.³⁶ In addition to depressive-like behavior, MS results in biochemical features of depression including neurochemical changes in the limbic system.²¹ Furthermore, treatment with antidepressants reverses both the behavioral and the biochemical changes.^{20,21} In the present study maternally

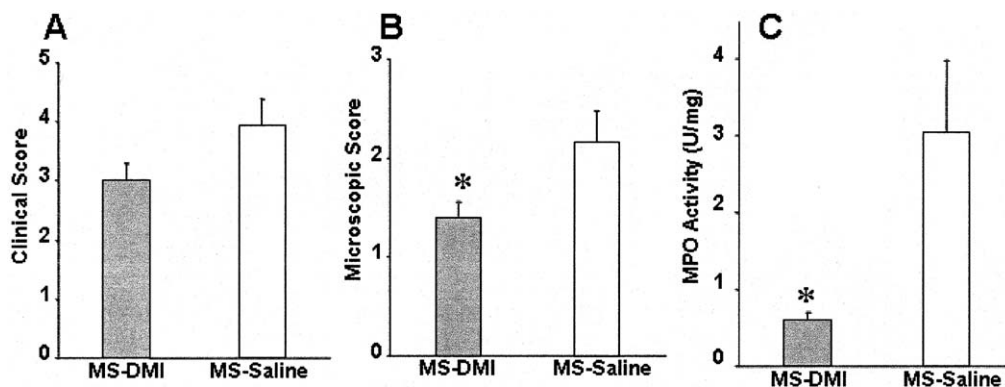


Figure 8. Colitis severity in MS mice treated with DMI after DSS colitis induction. (A) Clinical disease score. (B) Microscopic damage. (C) MPO activity in colonic tissue homogenates. Colitis was induced in MS-DMI and in MS-saline mice. Data are means \pm SEM (n = 8/group). *P < .05 compared with MS-saline mice.

separated mice showed depressive-like behavior, as reflected by increased immobility in the tail suspension test compared with US mice. Treatment with the antidepressant DMI reversed depressive-like behavior in MS mice because MS-DMI mice showed reduced immobility in the tail suspension test compared with MS mice treated with saline vehicle. Immobility in the tail suspension test is considered an index of depressive-like behavior because it is sensitive to antidepressants including DMI.²² Treatment of mice with DMI after MS has been shown also to ameliorate increased immobility of adult mice in other tests of depression, such as the forced swim test.²¹ Our findings show that MS mice show depressive-like behavior in adulthood and that this behavior is reversed by antidepressant treatment.

In the present study, MS mice also displayed several other behavioral and physiologic changes reflective of symptoms of depression. First, one characteristic of depression in human beings³⁷ and in animal models²⁰ involves an enhanced reactivity to stress, and this is reversed by antidepressants. In the present study, MS mice showed altered behavioral responses to novelty stress indicative of generalized stress vulnerability as compared with US mice. MS mice displayed a pattern of locomotion involving less distance traveled per movement bout as compared with US mice when exposed to the novel open field. This is consistent with the pattern of locomotion detected previously in MS adult rats on exposure to a novel environment.³⁸ MS mice also displayed reduced habituation of distance traveled and movement duration after exposure to a novel object as compared with US mice. Impaired habituation of novelty-induced locomotor activity has been reported previously in adult rodents after exposure to early life psychologic trauma similar to that of MS.³⁹ Second, in addition to the behavioral consequences of depression, MS mice also displayed enhanced body weights. Depression in late adolescence has been associated with subsequent development of obesity in adult human beings³⁹ and increased body weights in adult MS rodents, as compared with US rodents,⁴⁰ has been reported. The increased body weights observed in adult MS mice as compared with US mice, in this study, were not caused by increased food consumption because MS and US mice showed similar feeding patterns when tested at adulthood. The total amount of food consumed, the total number of feeding periods, and the amount of food consumed per feeding period were similar for adult MS and US mice. Increased hypothalamic-pituitary-adrenal axis activity has been shown in depressed patients⁴¹ and in adult rodents after MS.²⁰ It has been suggested that metabolic abnormalities including a hyperactive hypothalamic-pituitary-adrenal axis

may lead to altered body weight gain.⁴² Thus, we interpret the MS model in our study to reflect a paradigm of depression, and this is in agreement with the findings of others.^{20,21}

In the present study MS mice displayed no evidence of spontaneous inflammation or ill health; no changes were seen in histology, MPO activity, cytokine concentrations in the gut; and serum markers of inflammation were not increased. These results are consistent with that of 2 previous studies that did not detect spontaneous inflammation in adult MS rodents.^{43,44}

A recent study reported that mild depressive symptoms in human beings are associated with enhanced systemic inflammatory responses to immune challenge.¹⁴ The main finding in our study was that MS enhanced the severity of subsequent DSS colitis in adult mice. After exposure to DSS in adulthood, MS mice displayed enhanced severity of colitis, as assessed by greater weight loss, greater clinical disease scores, greater microscopic damage, and greater production of inflammatory markers including MPO, IL-1 β , and SAP, as compared with US mice. MS mice also displayed enhanced intestinal permeability as compared with US mice at adulthood. After intraluminal perfusion with a solution containing ⁵¹Cr-EDTA, jejunal loops isolated from MS animals displayed significantly greater radioactivity recovery in venous outflow as compared with that of US animals. We hypothesize that increased intestinal permeability facilitated the enhanced severity of subsequent DSS colitis. Decreased mucosal barrier function is believed to be an important pathogenic mechanism in the development of DSS colitis.⁴⁵ Increased mucosal permeability has been described as a very early change in acute DSS colitis⁴⁶ and factors that enhance intestinal barrier function have been shown to ameliorate acute DSS colitis.⁴⁵ However, the definitive role of increased intestinal permeability in enhanced DSS colitis cannot be examined at present owing to the lack of selective permeability-reducing agents. A previous study reported enhanced severity of trinitrobenzene sulfonic acid-induced colitis in adult MS rats.⁴⁷ However, the inflammatory stimulus trinitrobenzene sulfonic acid was administered to rats with pre-existing intestinal inflammation.⁴⁷ The enhanced vulnerability to colitis observed in MS mice in this study is more likely a long-term consequence of MS setting up a process leading to depressive-like symptoms because antidepressant treatment ameliorated the enhanced vulnerability to colitis.

MS mice treated with the antidepressant DMI showed decreased colitis severity as compared with MS mice given the saline vehicle. After exposure to DSS in adulthood, MS-DMI mice showed reduced microscopic disease

scores and reduced levels of the inflammatory marker MPO as compared with MS-saline mice. Certain antidepressants are known to have anti-inflammatory effects³⁴ and DMI may have an indirect anti-inflammatory effect through its modulation of noradrenaline bioavailability.⁴⁸ However, any anti-inflammatory effect of DMI is unlikely to have facilitated the reduced colitis vulnerability in MS-DMI mice because DMI treatment was concluded more than 3 weeks before DSS administration. Furthermore, DMI administration did not affect colitis severity in a separate set of adult US mice, as compared with US mice given saline. The amelioration of colitis vulnerability in MS mice by DMI likely is owing to reversal or arrest of a sensitization process triggered by MS that ultimately yields depressive-like symptoms.

It is believed that stressful or traumatic events occurring in early life, a critical period of brain development, result in dysregulation of stress-coping mechanisms that in turn predispose to affective disorders such as depression.⁴⁹ The corticotropin-releasing factor (CRF) system is an important mediator of the acute and long-term response to stress and later development of psychiatric illness.⁴⁹ Depression is associated with hyperactive CRF systems and a hyperactive hypothalamic-pituitary-adrenal axis, a classic marker of CRF system activity. Depressed patients show increased plasma and cerebrospinal fluid concentrations of cortisol, increased CRF in cerebrospinal fluid, increased numbers of CRF-containing neurons and CRF messenger RNA expression in the hypothalamus, and down-regulation of CRF receptors in the cerebral cortex and anterior pituitary.⁵⁰ Adult rodents show chronic hyperactivation of CRF systems and the hypothalamic-pituitary-adrenal axis and depressive-like behavior after MS.²⁰ Retrospective clinical and epidemiologic studies also have shown that a significant proportion of those who experienced childhood trauma developed hyperactivity of CRF systems and depression in adulthood.⁵¹ Furthermore, traditional antidepressants chronically administered to adult rats after neonatal MS have attenuated the hyperactivity of CRF systems and reversed the depressive-like behavior.²⁰ Traditional antidepressant drugs work in part by altering levels of biogenic amines including serotonin (5-HT), noradrenaline, and dopamine.⁴⁸ DMI belongs to the tricyclic antidepressant class and works by inhibiting noradrenaline reuptake into presynaptic nerve terminals.⁴⁸ Therefore, it is tempting to suggest that DMI attenuated the depressive-like behavior by dampening chronic hyperactivity of CRF systems after MS in our preparation.

The enhanced intestinal permeability shown by adult MS mice in this study is a likely consequence of the increased stress susceptibility associated with depression.

A previous study showed that MS induces depression and enhanced behavioral response to stress, which is associated with increased intestinal permeability in response to normally innocuous stressors in adult rodents.³⁸ Therefore, increased sensitivity to normally innocuous daily stressors may have led to enhanced intestinal permeability in adult MS mice in the present study.

The results of our study show that increased intestinal permeability alone is insufficient to cause inflammation. This is consistent with clinical studies that have reported increased permeability in the absence of overt intestinal inflammation.⁵² In a study showing a relationship between intestinal permeability and the subsequent development of IBD, it was evident that increased permeability can be present in the absence of overt inflammation.⁵³ Therefore, we hypothesize that increased intestinal permeability resulting from depression-induced stress vulnerability facilitated the development of enhanced colitis in response to DSS in maternally separated adult mice.

We attempted to reproduce the finding of enhanced susceptibility to colitis after MS in the DNB-induced colitis model. MS enhanced the susceptibility to subsequent DNB administration in adult mice. Mortality was observed in the MS group at 3 days after DNB administration, whereas no mortality was observed in the US group. Further experiments were not conducted using the DNB model because death is not a permissible end point at our institution. It is suggested that the mortality observed in the MS mice was caused by enhanced severity of colitis experienced by MS mice as compared with US mice. Previous studies from this laboratory have shown that the clinical signs of colitis including weight loss, diarrhea, and gross ulceration of the colon peak at day 3 after DNB exposure in C57BL/6 mice.⁵⁴ In addition, the acute inflammatory response, assessed in histologic sections of the colon as infiltration of the lamina propria and submucosa by neutrophils and assessed by MPO activity in colonic tissue homogenates, was maximal at day 3 after DNB administration.⁵⁴ Consequently, significant mortality was observed in C57BL/6 mice at 3 days after DNB administration.⁵⁴ Therefore, it is suggested that the increased mortality observed in MS mice, as compared with US mice in this study, resulted from an enhanced intestinal inflammatory response and systemic sequelae in MS mice after DNB administration. A breach of the intestinal barrier permitting mucosal entry of hapten-modified self-antigens and luminal bacteria is believed to be essential for the development of colitis induced by haptens including DNB.⁵⁵ Increased intestinal permeability, a factor that impairs epithelial barrier function, enhances the susceptibility to DNB colitis.³² It is concluded, therefore, that the impaired mucosal barrier

function, as indicated by increased intestinal permeability, detected in adult MS mice as compared with US mice contributed to the enhanced sensitivity to DNB shown by MS mice in this study.

In conclusion, depression in adult life after neonatal psychologic trauma may predispose to abnormal responses to intestinal inflammatory stimuli owing to enhanced stress reactivity in a model of depression. In individuals with a predisposition to IBD, depression may facilitate the development of overt inflammation and the expression of clinical disease through impaired intestinal barrier function. We have provided evidence that depression increases susceptibility to intestinal inflammation in mice and that decreased mucosal barrier function may be an underlying mechanism. These results have clinical relevance. First, our results should prompt an investigation into the relationship between depression and IBD. Second, our findings provide a rational basis for studying mucosal barrier function in depressed patients, without apparent gastrointestinal disease. Third, our results suggest that antidepressants may influence the natural history of IBD in patients with co-existing depression.

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