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

ASHWIN K. VARGHESE,* ELENA F. VERDÚ,* PREMYSL BERCIK,* WALIUL I. KHAN,* PATRICIA A. BLENNERHASSETT,* HENRY SZECHTMAN,† and STEPHEN M. COLLINS*

*Intestinal Diseases Research Program and †Department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario, Canada

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 depression 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 CD[8] 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 impairment[9] 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: ^{51}Cr-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.

0016-5085/06/$32.00
doi:10.1053/j.gastro.2006.02.007

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°C ± 5°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 × 40 × 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 × 450 × 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