Physiological stress exacerbates murine colitis by enhancing proinflammatory cytokine expression that is dependent on IL-18

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Physiological stress exacerbates murine colitis by enhancing proinflammatory cytokine expression that is dependent on IL-18. Am J Physiol Gastrointest Liver Physiol 301: G555–G564, 2011. First published June 30, 2011; doi:10.1152/ajpgi.00482.2010.—Psychological stress is an environmental factor considered to be a precipitating factor of inflammatory bowel disease. Interleukin (IL)-18 plays a role in stress-induced aggravation in some diseases. The aim of this study was to establish a model of murine colitis exacerbated by psychological stress and to clarify the role of IL-18 in this model. Male C57BL/6 mice and IL-18−/− mice were used for this study. The mice received dextran sulfate sodium (DSS) for induction of colitis. Some mice were exposed to psychological stress using a communication box. Body weight, colonic length, and histological inflammation were measured for assessment of colitis. Tumor necrosis factor (TNF)-α and IL-18 expression in the colon and IL-18 expression in the adrenal gland were analyzed using real-time PCR. The effect of anti-IL-18 antibody was also investigated. Effects of TNF-α and IL-18 on cytokine expressions were studied using the colonic epithelial cell line LS174T. Induction of psychological stress in DSS-treated wild-type mice significantly exacerbated colitis with enhanced expression of proinflammatory cytokines and IL-18. However, induction of psychological stress in DSS-treated IL-18−/− mice did not aggravate colitis compared with that in the IL-18−/− group given only DSS treatment. Stress-induced aggravation of colitis was ameliorated significantly by anti-IL-18 antibody treatment. IL-18 did not enhance TNF-α-induced expression of intercellular adhesion molecule-1 or IL-8 in LS174T. We established a model of colitis exacerbated by psychological stress. Psychological stress enhanced IL-18 expression and plays a proinflammatory role in stress-induced aggravation of colitis.

psychological stress; colitis; interleukin-18; mouse

THE PATHOGENESIS OF INFLAMMATORY bowel disease (IBD) is still unknown, and it has a complex multifactorial etiology comprising genetic (32) and environmental factors (2, 47) that are associated with dysregulation of the mucosal immune system. Psychological stress is an environmental factor that has long been suggested to contribute to the pathophysiology of IBD. Indeed, during the 1950s, ulcerative colitis was regarded as a psychosomatic disease (10). While clinical observations have provided anecdotal evidence (36, 37), there have been few prospective studies in which the involvement of stress in exacerbation or precipitation of inflammatory relapse in IBD patients was examined. A prospective cohort study showed that long-term perceived stress increases the risk of exacerbation of ulcerative colitis (19). Another recent study has suggested that several stressors can increase the rate of relapse in patients with IBD (21). In animal studies, it has been shown that captivity stress and readjustment to a novel social environment cause spontaneous colitis in cotton-topped tamarins (Sanguinus Oedipus) (8, 11). Repeated exposure to various stressors over a relatively short period, including restraint (12, 26), and a combination of cold and restraint stresses (33) also exacerbate colonic inflammation in rats. In addition, reactivation of completely resolved acute colitis after a combination of restraint and sonic stress has been reported (35). In agreement with the above-reported results, stress has been shown to affect the pathogenesis of diseases in immunological animal models (6, 17, 27, 45). However, although the involvement of somatic stress in colitis has been studied, the involvement of psychological stress has not been investigated.

Psychological and/or physical stresses affect host defenses comprising neuronal, endocrine, and immune systems (9, 18). A variety of cytokines [e.g., IL-1β, IL-6, and tumor necrosis factor (TNF)-α] are upregulated by stresses (9, 23, 53), suggesting that the cytokines are involved in interference with host defenses (38, 49, 54). Moreover, it has been reported that stress can modulate intestinal inflammatory responses through multiple routes, including neural and neuroendocrine pathways, the hypothalamus-pituitary-adrenal (HPA) axis, and the release of corticosteroid hormone (13). Communication between stress and the gut is via the HPA axis and sympathetic nervous system axis (13). Because stress has various effects on gastrointestinal functions (22, 43), including intestinal barrier function, luminal bacteria adherence, and mucosal immune functions, stress may directly and indirectly influence the balance of proinflammatory and anti-inflammatory cytokines in the intestine.

The pleiotropic interleukin (IL)-18 is thought to be one of the crucial mediators (15, 20, 42, 46, 50) since it activates various signal pathways, including those engaged in cell proliferation/survival (4, 28). Subsequent studies have demonstrated that IL-18 has multiple biological activities (7, 28, 29), including induction of Fas ligand, elevation of cytokitic activity of T cells (29), and production of Th2 cytokines (14, 52). IL-18 also activates Toll-like receptor 2 (3) and myeloid differentiation protein 88 (1). Thus, IL-18 is involved in the production of both Th1 and Th2 cytokines (7, 14, 28, 29). Conti et al. (5) showed that IL-18 mRNA is expressed in the adrenal gland in response to adrenocorticotropic hormone (ACTH) and cold stress. Sugama et al. (44) reported differential IL-18 promoter

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usage in the adrenal gland and immune cells with adrenal gland-specific expression of IL-18 mRNA induced by ACTH, suggesting that IL-18 may be induced in the adrenal gland during stress. These findings led us to speculate that IL-18 plays a pivotal role in the regulation of mucosal barrier functions under psychologically stressful conditions.

We hypothesized that psychological stress modulates colonic inflammation through IL-18. To determine the validity of our hypothesis, we investigated whether psychological stress exacerbates colonic inflammation and whether blockade of IL-18 modulates this inflammation.

MATERIALS AND METHODS

Animals. IL-18 gene knockout (IL-18<sup>−/−</sup>) mice, originally from the Jackson Laboratory (The Jackson Laboratory, Bar Harbor, ME) and wild-type mice on a C57Bl/6 background (Japan Clea, Tokyo, Japan) were maintained on a diet of standard laboratory chow (Oriental Yeast Manufacturing, Tokyo, Japan) and in specific pathogen-free conditions. Both wild-type and IL-18<sup>−/−</sup> mice were 8–10 wk of age, sex-matched, and housed four to five per cage, allowed free access to food and tap water, and were maintained in an animal colony at the National Defense Medical College (NDMC), according to the policies and recommendation of the NDMC Animal Care and Use Committee. This study protocol was approved by the Animal Ethical Committee of NDMC (no. 05093).

Induction of colitis. Dextran sulfate sodium (DSS, mol wt 40,000) was purchased from ICN Biochemicals (Cleveland, OH). DSS was dissolved in water and adjusted to a concentration of 3% (wt/vol). For induction of colitis, mice received DSS treatment for 5 days followed by 5 days with normal drinking water (24, 25).

Exposure to psychological stress. Psychological stress was produced using a communication box (Muromachi Kikai, Tokyo, Japan) that permitted one set of animals to receive a physical stressor in the form of a series of brief (10 s) electric shocks delivered using an electronic shock generator (31) over a period of 2 h. To induce psychological stress, DSS-treated mice or controls given only water were placed in compartments adjacent to those receiving the electric shock, and psychological stress was induced by their ability to view and hear the responses of the animals that received the physical stress (electric shock). To minimize distress, physically stressed animals were given only one 2-h session of electric shock and were then killed. DSS-treated mice and water controls exposed to psychological stress were exposed to different groups of physically stressed mice each day for five consecutive days after treatment with DSS or water. These groups of mice exposed to psychological stress were not given food or water during the time when mice of the physically stressed group received shock exposure. We note that the 5 days of repeated psychological stress was the minimum required to induce stress-associated weight loss in DSS-treated mice, and this same exposure did not reduce weight in water-treated mice. Mice that were not exposed to psychological stress were also deprived of food and water for 2 h per day for 5 days.

Assessment of colitis. Body weight was determined every 2 or 3 days. At the end of the study, the colon was removed and opened longitudinally. The length of colon was measured as a parameter for colonic inflammation. The colon was fixed in 10% buffered formalin. Tissues were embedded in paraffin, and they were stained with hematoxylin and eosin (H & E). Histological damage score was

![Fig. 1. Effects of dextran sodium sulfate (DSS) and psychological stress on percent change of body weight (A), colonic length (B), and grade of mucosal inflammation (C) in each group of wild-type mice. Water, a control group administered only water; Water + Stress, a group administered water and exposed to psychological stress; DSS, a group administered DSS; DSS + Stress, a group administered DSS and exposed to psychological stress. DSS treatment induced colitis, and exposure to psychological stress after DSS treatment aggravated colitis. Stress treatment alone did not induce colitis. Data are shown as means ± SE; n = 6 experiments. P < 0.05 vs. the water group (*) and vs. the DSS group (#).](http://ajpgi.physiology.org/)}
measured by the method of crypt damage and inflammation scoring by Williams et al. (51), ranging from 0 to 14. Total colitis is the sum of the four subscores. Inflammation severity was scored as follows: 0, none; 1, mild; 2, moderate; and 3, severe. Inflammation extent was scored as follows: 0, none; 1, mucosa; 2, mucosa and submucosa; and 3, transmural. Crypt damage was scored as follows: 0, none; 1, basal 1/3 damaged; 2, basal 2/3 damaged; 3, crypts lost; and 4, crypts and surface epithelium lost. Percent involvement was scored as follows: 0, 0%; 1, 1–25%; 2, 26–50%; 3, 51–75%; and 4, 75–100%. In some groups, the number of infiltrating immune cells was calculated and quantified per millimeter of muscularis mucosa.

Treatment with anti-IL-18 neutralizing antibody. In some groups, mice were treated with antibody daily from day 6 to day 10 for 5 days 3 h before psychological stress administration. Anti-IL-18 antibody (93–10C, rat IgG; MBL, Nagoya, Japan) or isotype- and species-matched Ig (rat IgG; Chemicon Intern, Temecula, CA) was administered intraperitoneally at the dose of 25 μg/mouse.

**Real-time PCR.** Intestinal mucosa was removed after mice were killed. Total mRNA was extracted by using the RNeasy Mini isolation kit (Qiagen). TaqMan reverse transcription PCR was performed in duplicate for each sample using the ABI PRISM 7000 Sequence Detector (Applied Biosystems). Primers and probes used in this study were purchased from Applied Biosystems: TNF-α (Mm00443258), IL-18 (Mm00434225), and IL-6 (Mm0044619).

**IL-18 expression in the adrenal glands.** Adrenal glands in the wild-type mice were removed after DSS treatment or exposure of psychological stress or both. IL-18 mRNA expression in the adrenal gland was analyzed using real-time PCR.

**IL-18, IL-18 receptor, and proinflammatory cytokine expression in the colonic epithelial cell line.** The human colon cancer cell line LS-174T was purchased from the American Type Culture Collection (ATCC CL188). Recombinant human IL-18 was from MBL, and recombinant human TNF-α was purchased from R&D (Minneapolis, MN). LS-174T was grown in MEM containing 10% FBS at 37°C,
95% air, and 5% CO₂. Cells were serum-starved and then treated with TNF-α at the concentration of 20 ng/ml or IL-18 at the concentration of 20 ng/ml for 6 h. Total RNA was harvested by using the RNeasy Mini isolation kit (Qiagen). Real-time quantitative RT-PCR was carried out by using the following primer and probe set: IL-18 (Hs01038788; Applied Biosystems), IL-18 receptor-1 (Hs00977691; Applied Biosystems), IL-8 (Hs00174103; Applied Biosystems), and intercellular adhesion molecule-1 [ICAM-1 (Hs00174103; Applied Biosystems)].

Statistical analysis. Data are expressed as means ± SE. Differences between groups were examined for statistical significance using one-way factorial ANOVA and Fisher’s protected least-significant difference test. P values of 0.05 or less were considered to be statistically significant. Statistical analyses were performed using the Statcel2 software (Addinsoft; OMS, Tokyo, Japan).

RESULTS

Exacerbation of DSS-induced colitis by psychological stress. Although psychological stress is thought to aggravate human colitis, there have been no studies to test whether psychological stress aggravates colitis in a murine model of colitis (20, 22). We investigated whether psychological stress aggravates DSS-induced colitis. Figure 1A shows the effects of DSS and psychological stress on percent change in body weight of wild-type mice. Mice in the water-administered plus psychological stress-exposed group gained weight throughout the study period. Mice in the water-administered plus psychological stress-exposed group gained weight throughout the study period. Mice in the DSS group and the DSS plus psychological stress group showed significantly higher body weight compared with that in the water group. The degree of body weight loss in the DSS plus psychological stress group was significantly higher than that in the DSS group. Figure 1B shows the effects of DSS and psychological stress on colonic length of wild-type mice. Colonic length in the DSS group and that in the DSS plus psychological stress group were significantly decreased compared with that in the water group. The degree of decrease in the DSS plus psychological stress group was significantly higher than that in the DSS group. Stress treatment alone did not decrease colonic length. Figure 2 shows a representative H & E section of the distal colon and grade of colonic inflammation in each group of wild-type mice. There were very few inflammatory cells and intact crypts in the colonic mucosa of mice in the water group (Fig. 2A) and water plus stress group (Fig. 2F). On the other hand, mild colitis characterized by an increased number of inflammatory cells, decreased number of crypts, and increased submucosal thickness was observed in the DSS without stress group (Fig. 2, B and C). Severe colitis characterized by total loss of crypt formation and numerous inflammatory cells in the lamina propria was observed in the DSS plus psychological stress group (Fig. 2, D and E). Figure 1C shows the grade of colonic inflammation in each group of wild-type mice. Histological damage score in the DSS group and that in the DSS plus psychological stress group were significantly increased compared with that in the water group. Histological damage score in the DSS plus psychological stress group was significantly higher than that in the DSS without psychological stress group.

Next we studied whether expression of proinflammatory cytokines in the colonic mucosa was involved in psychological stress-induced modification of colitis activity. Figure 3 shows relative mRNA expression levels of TNF-α (A) and IL-18 (B) in the colonic mucosa of each group of wild-type mice. Psychological stress treatment alone did not increase TNF-α expression levels. TNF-α expression levels in the DSS group and the DSS plus psychological stress group were increased significantly compared with that in the water group. In addition, TNF-α expression level in the DSS with psychological stress group was significantly higher than that in the DSS without psychological stress group. These results suggest that 1) TNF-α is involved in the pathogenesis of colitis, which is consistent with previous reports and 2) TNF-α is involved in the psychological stress-induced aggravation of colitis. IL-18 was also expressed in the colonic mucosa in the control water group. The level of IL-18 expression in the water plus stress group was significantly higher (2.7-fold higher) than that in the control water group. DSS treatment alone tended to increase IL-18 expression, although the increase was not significant. IL-18 expression level in the DSS plus psychological stress group was significantly increased compared with the levels in the control water group and the DSS group. These results suggest that IL-18 is involved in the psychological stress-induced aggravation of colitis.

Increase of IL-18 expression in adrenal glands after exposure to psychological stress. It is generally accepted that psychological stress increases IL-18 expression in adrenal glands through the HPA axis. We investigated whether IL-18 expression increased in our psychological stress model by using a communication box. Figure 4 shows relative mRNA expression of IL-18 in the adrenal glands of each group of wild-type mice. IL-18 was expressed in the control group. IL-18 expression levels in the psychological stress group and
the DSS plus psychological stress group were increased significantly compared with the level in the control group. IL-18 expression in the DSS group was also increased compared with that in the control group. However, the increase was not significant. The increase in the DSS plus psychological stress group was significant even compared with the expression in the DSS group.

Exacerbation of DSS colitis by psychological stress was not observed without IL-18. We showed that psychological stress aggravated colitis with enhanced expression of proinflammatory cytokines and IL-18. Next, we tried to clarify whether IL-18 was involved in this process. For this purpose, we used IL-18-deficient mice. Figure 5A shows the effect of DSS and psychological stress on percent change in body weight of IL-18 knockout mice. The IL-18 knockout mice in the DSS group and the DSS plus psychological stress group showed significant weight loss compared with the weight in the water group. However, the degree of body weight loss in the DSS plus psychological stress group was almost the same, and stress-induced body weight loss was not observed in IL-18 knockout mice, suggesting that psychological stress-induced aggravation of colitis was IL-18-dependent. Figure 5B shows the effect of psychological stress in IL-18 knockout mice on DSS colitis determined by colonic length. Colonic lengths in the DSS group and the DSS plus psychological stress group were decreased significantly compared with that in the water group. Shortening of the colon was almost the same in the DSS group and the DSS plus psychological stress group, and psychological stress-induced aggravation of DSS colitis was not observed. Figure 6 shows a representative H & E section of the distal colon and grade of colonic inflammation in each group of IL-18 knockout mice. There were few inflammatory cells and intact crypts in the colonic mucosa of IL-18 knockout mice in the water group. On the other hand, mild colitis was observed...
in IL-18 knockout mice treated with DSS, and the colitis was characterized by increased inflammatory cells, a decrease in the number of crypts, and an increase in thickness of submucosal and muscularis layers. However, induction of psychological stress in IL-18 knockout mice did not enhance DSS-induced colitis, and the activity of colitis remained mild. Crypt formation was preserved in the DSS plus stress group of IL-18 knockout mice but had completely disappeared in the same treatment group of wild-type animals (Fig. 1C). Figure 5C shows the grade of colonic inflammation in each group of IL-18 knockout mice. Histological damage scores in the DSS group and the DSS plus psychological stress group were increased significantly compared with that in the water group. The IL-18 knockout mice in the DSS plus psychological stress group did not show further aggravation compared with the histological damage score in the DSS group of IL-18 knockout mice. We showed that psychological stress-induced aggravation of colitis was accompanied by an increase in TNF-α. We next clarified whether psychological stress increased TNF-α in DSS-induced colitis in the absence of IL-18. Figure 7 shows relative mRNA expression of TNF-α in the colonic mucosa of each group of IL-18 knockout mice. Interestingly, psychological stress-induced enhancement of TNF-α expression in DSS-treated mice was not observed in IL-18 knockout mice, suggesting that TNF-α is involved in stress-induced immune imbalance.

**Effect of blocking of IL-18 by anti-IL-18 antibody on psychological stress-induced aggravation of DSS colitis.** To further evaluate the role of IL-18 in psychological stress-induced aggravation of DSS colitis, we divided wild-type mice into the
following three groups: DSS plus psychological stress with IL-18 antibody treatment group, DSS plus psychological stress with control antibody treatment group, and water-administered control group. We treated mice with antibodies 3 h before exposure to psychological stress from day 6 to day 10. In the DSS plus psychological stress with control antibody treatment group, body weight decreased during DSS treatment (day 1 to day 6) and continued to decrease even during the stress exposure period (day 6 to day 11). On the other hand, in the DSS plus psychological stress with anti-IL-18 antibody treatment group, body weight started to increase after anti-IL-18 antibody treatment, and body weight loss was significantly less than that in the DSS plus psychological stress with control antibody treatment group (Fig. 8A). Shortening of colonic length was also less in the DSS plus psychological stress with IL-18 antibody treatment group than in the DSS plus psychological stress with control antibody treatment group (Fig. 8B). Grade of colonic inflammation was also less in the DSS plus psychological stress with IL-18 antibody treatment group than in the DSS plus psychological stress with control antibody treatment group (Fig. 8C). The level of expression of proinflammatory cytokines, TNF-α and IL-6, was also lower in the DSS plus psychological stress with IL-18 antibody treatment group than in the DSS plus psychological stress with control antibody treatment group (Fig. 8D). The number of infiltrating cells in the colonic mucosa was also less in the DSS plus psychological stress with IL-18 antibody treatment group than in the DSS plus psychological stress with control antibody treatment group (Fig. 8E). These results directly showed that neutralization of IL-18 ameliorated psychological stress-induced aggravation of DSS colitis.

TNF-α increased expression levels of IL-18 receptor and proinflammatory cytokines in a colonic epithelial cell line. Finally, we investigated whether epithelial cells expressed IL-18, IL-18 receptor, and proinflammatory cytokines that are known to be induced through the NF-κB pathway (IL-8 and ICAM-1). In addition, we investigated how the cells were affected by TNF-α and/or IL-18 treatments. LS-174T cells expressed mRNAs of IL-18, IL-18 receptor, IL-8, and ICAM-1 in the control condition. TNF-α, which is known to activate the NF-κB pathway, significantly increased the expression levels of IL-8 and ICAM-1 mRNAs, suggesting that this cell line is useful for evaluating the effect of IL-18 on expression of proinflammatory cytokines. However, treatment with IL-18 alone did not enhance the expression of IL-8 or ICAM-1. Because TNF-α increased IL-18 receptor expression, we treated cells with both TNF-α and IL-18 to highlight the role of IL-18. However, addition of IL-18 to TNF-α did not enhance IL-8 or ICAM-1 expressions, suggesting that proinflammatory cytokines from epithelial cells were not involved in IL-18-induced aggravation of colitis (Fig. 9).

**DISCUSSION**

Psychological stress is an environmental factor that has long been suggested to contribute to the pathophysiology of IBD. A recent study has suggested that several stressors can increase the rate of relapse in patients with IBD (21). It has been shown that captivity stress and readjustment to a novel social environment cause spontaneous colitis in cotton-topped tamarins (8, 11). In the present study, we showed for the first time by using a mouse model of colitis that addition of psychological stress to colonic inflammation caused aggravation of colitis. Mice were exposed to various emotional conditioned stimuli (psychological stress) by watching and hearing the stress responses of physically stressed mice that received electric shock. This model made it possible to clarify the mechanism of psychological stress-induced aggravation of colitis by using genetically engineered mice. We treated mice with psychological stress after the DSS drinking period to avoid an affect of psychological stress on the quantity of DSS consumption. In addition, we treated mice with a lower concentration of DSS for induction of colitis to distinguish the effect of psychological stress on colitis. Almost all parameters, including body weight loss, shortening of colonic length, and histological inflammation, were aggravated by psychological stress.

The mechanism of modulation of stress to the colonic immune system is not clear. However, several pathways have been suggested: 1) the HPA axis pathway, 2) the systemic nervous system axis pathway, and 3) the opioid receptor system pathway. We paid attention to the HPA axis in these pathways. We focused on IL-18 because 1) IL-18 mRNA is expressed in response to a stressor in the adrenal gland through the HPA axis (5), 2) stress-induced IL-18 enhances production of proinflammatory cytokines (40), and 3) IL-18 is involved in the pathophysiology of stress-induced histamine-dependent gastric injury (39). In this study, we treated mice with an anti-IL-18 neutralizing antibody during the period of exposure to stress, which was after DSS drinking treatment. Anti-IL-18 antibody significantly ameliorated stress-induced aggravation of colitis compared with the control antibody-treated group. In addition, stress-induced aggravation of colitis was not observed in IL-18−/− mice. Taken together, these results suggested that stress-induced aggravation of colitis was IL-18-dependent. In this study, psychological stress enhanced expression of IL-18 in the adrenal gland, suggesting that the HPA axis pathway was activated by communication box-induced psychological stress. Interestingly, enhanced expression of IL-18 was observed not only in the adrenal gland but also in the colonic mucosa. Recently, it has been reported that isolation stress enhanced expression of IL-18 in the murine rectum (30).
Kanai et al. (16) and Siegmund et al. (41) reported differential IL-18 promoter usage in the adrenal gland and immune cells with adrenal gland-specific expression of IL-18 mRNA by ACTH. It has been reported that both epithelial cells and macrophages expressed IL-18 in the colonic mucosa of murine colitis (16, 41). Because we did not use organ-specific conditional knockout IL-18 mice, it is not known from which organ and which kind of cells that IL-18 was responsible for aggravation of stress-induced colitis. The source of IL-18-producing cells in the colonic mucosa and the mechanism of regulation have yet to be clarified. However, IL-18 induction only by psychological stress in the rectal mucosa suggests that the large intestine is the vulnerable organ for stress-induced aggravation of inflammatory response. To clarify possible involvement of IL-18 in epithelial cells in the stress-induced immune response, we investigated the expression of IL-18, IL-18 receptor, and proinflammatory cytokines that are known to be induced through the NF-κB pathway in an epithelial cell line in vitro. Epithelial cells expressed both IL-18 and IL-18 receptor. However, treatment of epithelial cells with IL-18 did not enhance the expression of IL-8 or ICAM-1. These results suggested that epithelial cells may be a source of IL-18 but unlikely a target for its action.

In our study, expression of TNF-α was increased by DSS treatment, and psychological stress further increased TNF-α expression. The absence of IL-18 completely blocked the psychological stress-induced increase in TNF-α expression. IL-18 enhances the production of both Th1 and Th2 cytokines (7, 14, 28, 29). IL-18 aggravates TNF-α-induced hepatic injury in mice (48). It has been reported that IL-18-induced TNF-α
expression was observed in CD3+/CD4+ cells but not in CD14+ macrophage lineage cells and that this induction was inhibited by IL-10 (34). It is generally accepted that a relative decrease in IL-10 is responsible for the pathogenesis of IBD. Thus, stress-induced IL-18 production might play a proinflammatory role synergistically with a decrease in IL-10.

Previously, Kanai et al. (15, 16) showed the involvement of IL-18 in murine colitis. In our study also, DSS treatment alone enhanced expression of IL-18 in the murine colon. Because we induced mild colitis, the level of IL-18 expression induced by DSS treatment was comparable to that induced by psychological stress alone. It is possible that DSS treatment itself was a stressor to induce IL-18. Alternatively, it is possible that IL-18 was upregulated by another cytokine indirectly. In this study, the degree of DSS-induced colitis in wild-type mice and that in IL-18−/− mice was comparable. However, stress-induced aggravation was completely blocked in the absence of IL-18. In addition, systemic inhibition by an IL-18 neutralizing antibody ameliorated stress-induced aggravation of colitis. Collectively, the results suggest that IL-18 is involved in the pathophysiology of proinflammatory response in colonic inflammation under psychologically stressful conditions.

GRANTS

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DISCLOSURES

Competing interests: none.

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