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Psychological stress promotes neutrophil infiltration in colon tissue through adrenergic signaling in DSS-induced colitis model

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ABSTRACT

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory condition. Psychological stress has been postulated to affect the clinical symptoms and recurrence of IBD. The exact molecular mechanisms are not fully understood. In the present study, we demonstrate that psychological stress promotes neutrophil infiltration into colon tissues in dextran sulfate sodium (DSS)-induced colitis model. The psychological stress resulted in abnormal expression of the proinflammatory cytokines (IL-1β, IL-6, IL-17A, and IL-22) and neutrophil chemokines (CXCL1 and CXCL2) and overactivation of the STAT3 inflammatory signaling pathway. Under chronic unpredictable stress, the adrenergic nervous system was markedly activated, as the expression of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, in bone marrow and colonic epithelium was enhanced, especially in the myenteric ganglia. The β-AR agonist isoproterenol mimicked the effects of psychological stress on neutrophilia, neutrophil infiltration, and colonic damage in DSS-induced colitis. The β1-AR/β2-AR inhibitor propranolol reduced the numbers of the neutrophils in the circulation, suppressed neutrophil infiltration into colonic tissues, and attenuated the colonic tissue damage promoted by chronic stress. Propranolol also abolished stress-induced upregulation of proinflammatory cytokines and neutrophil chemokines. Our data reveal a close linkage between the β1-AR/β2-AR activation and neutrophil trafficking and also suggest the critical roles of adrenergic nervous system in exacerbation of inflammation and damage of colonic tissues in experimental colitis. The current study provides a new insight into the mechanisms underlying the association of psychological stress with excessive inflammatory response and pathophysiological consequences in IBD. The findings also suggest a potential application of neuroprotective agents to prevent relapsing immune activation in the treatment of IBD.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing, and remitting disease characterized by chronic irritation and inflammation in the gastrointestinal tract. The causes leading to chronic intestinal inflammation in IBD remain elusive. A defective mucosal immune mechanism has been indicated in the pathogenesis of chronic intestinal inflammation (Goldberg et al., 2015; Maloy and Powrie, 2011). Emerging evidence suggests that stress may affect clinical symptoms of idiopathic IBD (such as ulcerative colitis and Crohn's disease) (Goodhand and Rampton, 2008; Mawdsley and Rampton, 2005).

The previous studies showed that experimental psychological stress contributed to both the initiation and reactivation of gastrointestinal inflammation in animal models of colitis. For example, chronic psychosocial stress increases the severity of 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced colitis in rat. Reactivation of mucosal inflammation occurred in rats, which had recovered from TNBS-induced colitis, in response to restraint stress (Mawdsley and Rampton, 2005). Clinical studies revealed

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that psychological stress exacerbated the disease course and caused relapses of IBD patients. On the other hand, IBD was also associated with an increased risk of primary psychiatric diseases such as depression and anxiety disorders (Gerbarg et al., 2015; Reichmann et al., 2015). However, the underlying mechanisms have not been fully understood.

Stress response is natural response or reaction to environmental demands or pressures. In response to chronic stressors, autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis, which is the central stress response system, are activated, resulting in sustained release of catecholamine [such as epinephrine and norepinephrine (NE)] from sympathetic neurons and adrenal medulla and cortisol from adrenal cortex (Chrousos, 2009). Over-release of a variety of neurotransmitters (such as catecholamines and vasoactive intestinal peptide) influences the activities of lymphocytes, macrophages, and neutrophils. Immune cells express adrenergic receptors (ARs), through which locally released NE or circulating catecholamines regulate the production of cytokines and antibodies and the functional activities of immune cells (Irwin and Cole, 2011).

Multiple lines of evidence indicate that NE and epinephrine inhibit the production of pro-inflammatory cytokines but stimulate the production of anti-inflammatory cytokines, resulting in a selective suppression of Th1 response and promotion of Th2 polarization (Evans et al., 2015). However, catecholamines may also boost regional immune response by inducing the production of pro-inflammatory cytokines under certain conditions. In the patients with rheumatoid arthritis, the inhibitory effects of catecholamines on IFN-γ production or Th2 shift were impaired or lost (de Brouwer et al., 2014; Straub, 2014). In IBD, abnormal amplification and persistence of inflammation by chronic stress can cause severe tissue injuries (Mawdsley and Rampton, 2005). The triggering factors initiating the inflammatory response are mostly unknown.

The present study set out to explore the mechanistic basis of psychological stress-induced colonic tissue injuries in DSS-induced colitis model. We hypothesized that the activation of the stress-associated signaling lowers the threshold for triggering an inflammatory response in acute colitis, resulting in exacerbated colon mucosal damage. Therefore, we investigated the effects of chronic stress on the expression of proinflammatory cytokines and on inflammatory cell recruitment/infiltration in colonic tissues. We further explored the stress-triggered signaling pathways that are involved in the pathogenesis of IBD.

2. Materials and methods

2.1. Mice

Seven to eight-week-old male C57BL/6 mice were housed in a pathogen-free facility and the animal studies were approved by the Animal Care and Use Committee of Institute of Basic Medical Sciences.

2.2. Chronic restraint stress (CRS) and chronic unpredictable stress (CUS)

After acclimation for one week, the mice were individually placed in a well-ventilated and transparent plastic mouse restraint system (Thaker et al., 2006) for four hours per day for consecutive seven days. The mouse restraint system was cleaned and sterilized between each restraint cycles. Food- and water-deprived but not restrained mice were used as control animals, since stressed mice in the restraint system did not have access to food and water during this time period.

For CUS, the mice were exposed to chronic variable stressors, including cage tilt, isolation, crowding, rapid light-dark changes, damp bedding, and overnight illumination (Supplementary Table 1). All stressors were randomly shuffled in consecutive five days. The detailed procedure was described previously (Heidt et al., 2014).

2.3. Induction of colitis

Experimental acute colitis was induced by giving mice *ad libitum* access to drinking water containing 2.5% dextran sulfate sodium (DSS, MP Biomedicals, US) for seven days. To investigate the effects of stress on the severity of experimental colitis, DSS was added to the drinking water after exposure to CRS or CUS for five days. To determine the effects of catecholamines and activation of stress-related signaling pathways, mice were injected intraperitoneally with 10 mg/kg/day isoproterenol (ISO), 10 mg/kg/day propranolol (Sigma-Aldrich, UK), 5 mg/kg/day the beta 3-selective adrenoceptor antagonist (SR 59230A, Sigma-Aldrich, UK), or the same volume of solvent (Azhdarinia et al., 2013; Heidt et al., 2014; Lin et al., 2015; Thaker et al., 2006).

2.4. Flow cytometry

Peripheral blood samples were collected from the tail vein of the mice into tubes containing heparin. The blood cells were resuspended in PBS containing 1% bovine serum albumin (BSA) and then stained with fluorescein isothiocyanate-conjugated rat anti-mouse CD11b, APC-conjugated rat anti-mouse Ly6G, and PE-conjugated rat anti-mouse Ly6C (Biolegend, US) for 30 min in dark at room temperature. Then the samples were treated with erythrocyte Lysis buffer (BD, Bioscience, UK) following the manufacturer's instructions. After washing with PBS twice, the $Ly6C^+$ cells were gated and analyzed for the expression of CD11b and Ly6G on a FACScan flow cytometer using CellQuest software (BD, Bioscience, UK). The experiments were repeated at least twice.

For evaluating the infiltration of neutrophils in colon tissues, the animals were perfused with saline to wash out circulating blood cells under anesthesia. After perfusion, the colon tissues were collected, carefully cleaned in ice-cold PBS, and then mechanically chopped. The colon tissues were digested for 30 min at 37 °C in 1 mg/ml collagenase type IV (Gibco, US) and 1 mg/ml dispase II (Sigma-Aldrich, UK) in serum-free RPMI 1640 medium. The enzyme activities were neutralized by addition of cold RPMI 1640 medium containing 8% fetal bovine serum (FBS). The suspension was dispersed through a 150 μm cell strainer. The sing-cell suspensions were stained with PerCP-conjugated rat anti-mouse CD45, fluorescein isothiocyanate-conjugated rat anti-mouse CD11b, and APC-conjugated rat anti-mouse Ly6G (Biolegend, US). The $CD45^+$ cells were gated and analyzed for the expression of CD11b and Ly6G. The experiments were repeated at least twice.

2.5. Bacterial translocation

Bacterial translocation assay was performed as previously described (Yang et al., 2002). Briefly, the skin was cleaned, the abdominal cavity opened, and the viscera exposed using sterile techniques. The mesenteric lymph nodes were removed, weighed, and homogenized. The homogenates (100 μl) were plated onto Luria-Bertani agar (OXOID) and incubated at 37 °C under aerobic conditions for 24 hours. The colonies were counted and results expressed as colony-forming units (CFU) per gram of tissue.