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Interleukin 19 reduces inflammation in chemically induced experimental colitis

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ABSTRACT

Inflammatory bowel disease results from chronic dysregulation of the mucosal immune system and aberrant activation of both the innate and adaptive immune responses. Interleukin (IL)-19, a member of the IL-10 family, functions as an anti-inflammatory cytokine. Here, we investigated the contribution of IL-19 to intestinal inflammation in a model of T cell-mediated colitis in mice. Inflammatory responses in IL-19-deficient mice were assessed using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) model of acute colitis. IL-19 deficiency aggravated TNBS-induced colitis and compromised intestinal recovery in mice. Additionally, the exacerbation of TNBS-induced colonic inflammation following genetic ablation of IL-19 was accompanied by increased production of interferon-gamma, IL-12 (p40), IL-17, IL-22, and IL-33, and decreased production of IL-4. Moreover, the exacerbation of colitis following IL-19 knockout was also accompanied by increased production of CXCL1, G-CSF and CCL5. Using this model of induced colitis, our results revealed the immunopathological relevance of IL-19 as an anti-inflammatory cytokine in intestinal inflammation in mice.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are the two types of inflammatory bowel diseases (IBDs) and are characterized by dysregulated intestinal inflammation and mucosal tissue damage in parts of the gastrointestinal tract [1]. The maintenance of intestinal homeostasis is complex and involves interactions among the intestinal microflora, epithelium, and host immune system. Interleukin (IL)-10, a well-known anti-inflammatory and immunosuppressive cytokine, has shown promise in clinical trials for the treatment of IBDs. Biologically based therapies, such as recombinant IL-10, can reduce symptoms of these disorders [2]. Consistent with this, IL-10-deficient mice spontaneously develop colitis, indicating that IL-10 is critical for colonic protection [3].

IL-19 is a member of the IL-10 family, which also includes IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29 [4], and has been shown to be expressed by epithelial cells, macrophages, and B-cells [5]. Furthermore, in vitro studies have shown that lipopolysaccharide can stimulate human monocytes to upregulate the expression of *IL-19* mRNA [6] and that recombinant IL-19 can activate mouse monocytes, stimulating the production of pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor (TNF)-α [7]. Recent studies have shown that IL-19 is associated with the development of T-helper (Th) 2 responses [8,9] and the pathogenesis of psoriasis [10–12]. However, little is known about the exact biological role of IL-19 in the regulation of intestinal inflammation. Interestingly, our previous studies showed that IL-19-knockout (KO) mice are more susceptible to experimental acute colitis induced by dextran sulfate sodium (DSS), and this increased susceptibility is correlated with the accumulation of macrophages and the increased production of interferon (IFN)-γ, IL-1β, IL-6, IL-12, TNF-α, and CXCL1. The finding that IL-19 restrains pathogenic innate immune responses in the colon suggests that the selective targeting of IL-19 may be an effective therapeutic approach in the treatment of human IBD [13].

Clinically, CD is characterized by transmural, discontinuous inflammation, which is associated with a type-1 response mainly driven by IL-12 and IFN-γ [14], while UC involves the superficial mucosal and submucosal layers of the colon and is driven by type-2 cytokines, such as IL-4, IL-5, and IL-13 [15]. The 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis model shows a clear type-1 phenotype [16], whereas the oxazolone-induced colitis model is useful for the study of the type-2 inflammatory response [17,18].

In this study, to determine whether the role of IL-19 in the protection from colonic inflammation was exclusive to the DSS-induced colitis model, we investigated the role of IL-19 in TNBS-induced colitis. Our data demonstrated that IL-19 had anti-inflammatory effects in this model of chemically induced experimental colitis.

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2. Materials and methods

2.1. Mice

IL-19-KO mice were obtained from Regeneron Pharmaceuticals, Inc. (Tarrytown, NY, USA) as described previously [19]. C57BL/6-IL-19 mice were backcrossed onto the Balb/c genetic background for at least 10 generations. Balb/c-IL-19 heterozygous mice were intercrossed to generate mutant and control mice. Age-matched mice (10–15 weeks old) were used in all experiments. We studied male mice, and only data of body weight change was obtained from both male and female mice. All procedures used in this study complied with institutional policies of the Osaka Prefecture University Animal Care and Use Committee.

2.2. TNBS-induced colitis

TNBS was prepared in a 50% ethanol solution at a final concentration of 25 mg/mL. Colitis was induced by intrarectal administration with 100 μL of TNBS solution using a plastic catheter. Control mice were administered a 50% ethanol solution without TNBS using the same technique. Body weights were monitored daily.

2.3. Histology

The distal colon was fixed with 10% neutral buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE). The severity of intestinal inflammation was graded using a previously described system [20] with minor modifications. The score ranges from 0 to 9 (total score), which represents the sum of scores from 0 to 3 for infiltration as follows: 0, no infiltration; 1, low level; 2, moderate level; 3, high level, 0 (none) to 2 (positive) for serositis, 0 (none) to 2 (positive) for ulcer and 0 (none) to 2 (positive) for transmural necrosis. All sections were scored in a blinded fashion by a pathologist.

2.4. RNA isolation, microarray analysis, and quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was isolated from the distal colon as previously described [21], with minor modifications. Comprehensive DNA

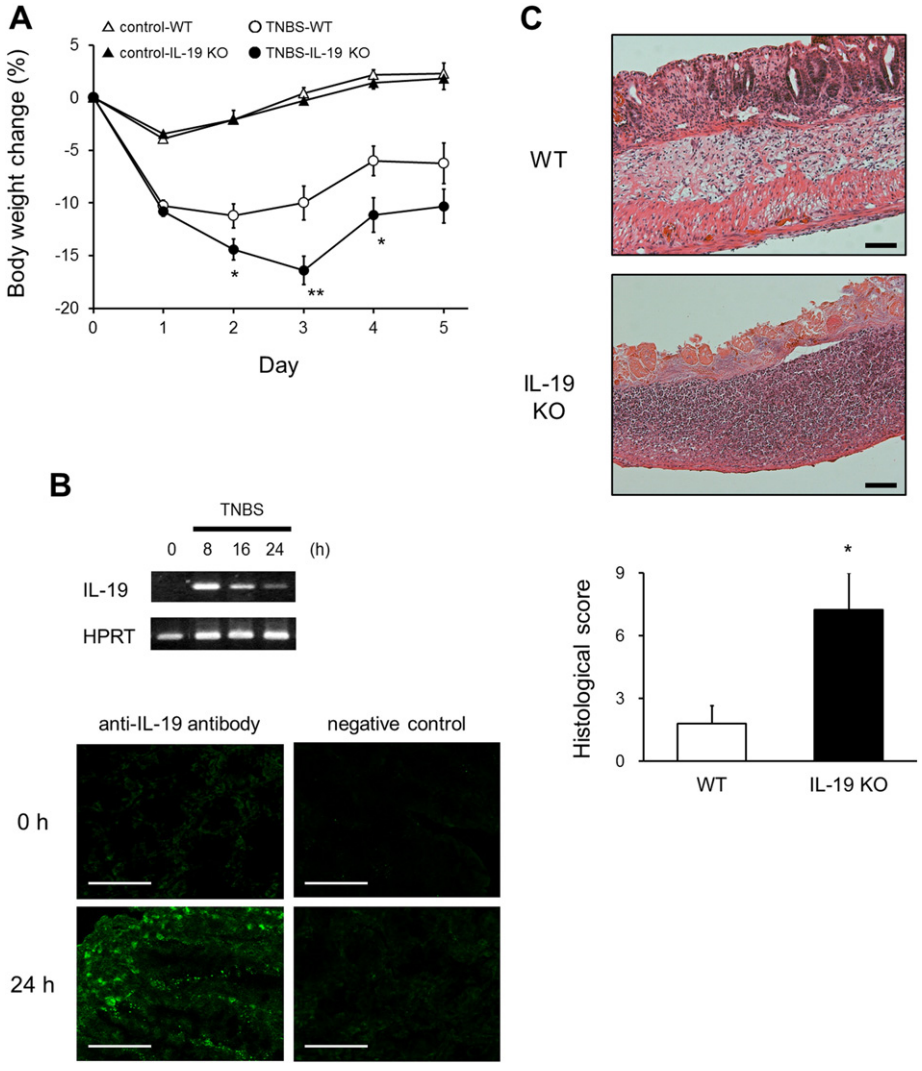

Fig. 1. Increased susceptibility of IL-19-KO mice to TNBS-induced colitis. A) The percent weight loss of WT (n = 12) and IL-19-KO (n = 17) mice was monitored daily. B) Expression of *IL-19* mRNA in the distal colon of WT mice (n = 3) at 8, 16, and 24 h after TNBS administration. Immunohistochemical staining of tissue sections in the distal colon of WT mice at 0 and 24 h after TNBS administration. Tissue sections were stained for IL-19 (green). Images shown are representative of three experiments. Scale bar, 50 μm. C) On day 3 post-TNBS administration, mice were euthanized, and their distal colons were removed, fixed, sectioned, and stained. Data include WT (n = 4) and IL-19-KO (n = 4) mice. H&E staining of representative sections of the distal colon. Scale bar, 100 μm. Histological scores for the distal colon were determined. $^{*}P < 0.05$, $^{**}P < 0.01$.