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Interleukin-25 Inhibits Interleukin-12 Production and Th1 Cell-Driven Inflammation in the Gut

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Background & Aims: **During the pathogenesis of Crohn's disease (CD), interleukin (IL)-12, a cytokine produced by mucosal CD14$^+$ monocyte-like cells, promotes tissue-damaging T helper cell (Th) 1-mediated inflammation through mechanisms that are not fully understood. IL-25 promotes Th2 cell responses by activating major histocompatibility complex class II-positive non-T and non-B cells. Because Th1 and Th2 cells, and the cytokines they release, are often mutually antagonistic, we examined whether IL-25 affects IL-12 production or Th1 cell-mediated inflammation in the gut.** ***Methods:*** **Studies were performed using colonic samples from patients and mice with peptidoglycan (PGN)-, 2,4,6-trinitrobenzenesulphonic acid (TNBS)-, or oxazolone-induced colitis. IL-25 receptor (IL-25R) levels were evaluated in intestinal lamina propria mononuclear cells by flow cytometry, and IL-25 levels were measured by real-time polymerase chain reaction, immunoblotting, and immunohistochemistry. Mucosal CD14$^+$ cells from patients with CD were incubated with IL-25 and/or lipopolysaccharide or PGN. Mice were injected with IL-25, and some mice first received injections of an IL-13 blocking antibody. Cytokines were quantified by real-time polymerase chain reaction and enzyme-linked immunosorbent assay.** ***Results:*** **CD14$^+$ cells from the mucosa of CD patients expressed IL-25R and responded to IL-25 by decreasing the synthesis of IL-12 and IL-23. IL-25 prevented PGN-induced colitis in mice. IL-25 induced IL-13 production in the colon, but IL-13 was not required for suppression of PGN colitis. IL-25 ameliorated TNBS- and oxazolone-colitis. Patients with CD or ulcerative colitis produced significantly less IL-25 compared with controls.** ***Conclusions:*** **IL-25 inhibits CD14$^+$ cell-derived cytokines and experimental colitis. IL-25 could be a useful treatment of CD and ulcerative colitis.**

In the normal gut, mononuclear phagocytes represent a nonreactive and tolerogenic population, as indicated by the reduced expression of several activation-associated surface molecules and their inability to respond to commensal bacterial and inflammatory stimuli.[1,2] In contrast, in inflammatory bowel diseases (IBD), this state of "hyporesponsiveness" is disturbed, and phagocytes produce large amounts of proinflammatory molecules that promote differentiation and activation of effector T cells, which drive mucosal damage. This occurs, for example, in Crohn's disease (CD) tissue, where a pathogenic T helper cell (Th) 1 type-associated immune response is thought to be driven by interleukin (IL)-12, a heterodimeric cytokine composed of 2 subunits: p40 and p35.[3] Differences in the functional properties of phagocytes between normal and CD gut are in part due to the marked accumulation of blood CD14$^+$ cells in the inflamed mucosa of CD patients.[4] These cells, in contrast to resident macrophages, are capable of synthesizing inflammatory molecules in response to stimulatory signals.[5] Consistently, it has been shown that, in CD, IL-12 is made by CD14$^+$ monocyte-like cells.[6] Factors/mechanisms that control CD mucosal CD14$^+$ cell function and IL-12 production have not been extensively investigated.

IL-25 (also known as IL-17E) is a member of the IL-17 cytokine gene family and is made by several cell types, including T lymphocytes, mast cells, eosinophils, and basophils.[7] IL-25 facilitates pathogenic Th2 cell responses, causes epithelial cell hyperplasia, and enhances the recruitment of inflammatory cells into inflamed tissues.[8–11] Moreover, Owyang et al[12] showed that IL-25-null mice develop a severe intestinal inflammation characterized by exaggerated production of IL-17 and interferon (IFN)-γ following chronic infection with *Trichuris muris*, thus suggesting that IL-25 can also control the outcome of Th1/Th17 cell responses.[12] Induction of IL-13 seems to be the mechanism by which IL-25 inhibits the development of Th17-mediated pathology.[13]

IL-25 biologic activity on target cells requires the expression of a transmembrane receptor (IL-25R), also called IL-17RB, IL-17R homolog 1, or IL-17RA.[14,15] The cellular targets of IL-25 are not yet entirely known, but

Abbreviations used in this paper: CD, Crohn's disease; LPMC, lamina propria mononuclear cells; PGN, peptidoglycan; Th, T helper cell; TNBS, 2,4,6-trinitrobenzenesulphonic acid; UC, ulcerative colitis.

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studies in recombinase-activating gene (RAG) knockout mice have shown that IL-25 acts preferentially on a non-B/non-T-cell type expressing high levels of major histocompatibility complex class II and low levels of CD11c.[9] It is thus conceivable that IL-25 may regulate the activity of phagocytes and interfere with mechanisms that control Th cell differentiation. Because Th1, Th2, and Th17 cells, and the cytokines they release, are often mutually antagonistic,[16] we examined whether IL-25 controls IL-12 production and Th1 cell responses in the gut.

Materials and Methods

Mucosal Samples

Colonic mucosal biopsy specimens were taken from 16 patients with CD and 8 patients with ulcerative colitis (UC). Surgical specimens from 13 CD patients and 4 UC patients undergoing resection for a chronically active disease poorly responsive to medical treatment were also analyzed. Twenty-four patients with CD and 9 patients with UC were receiving corticosteroids and mesalazine, whereas the remaining patients were untreated. Additional biopsy samples were taken from 5 patients with IBD (2 CD and 3 UC) before and after treatment with corticosteroid and azathioprine. Colonic samples were also taken from 4 patients with diverticulitis. Normal controls included samples taken from 17 patients with irritable bowel syndrome (IBS) and from the macroscopically and microscopically unaffected areas of 8 patients with colon cancer.

Lamina Propria Mononuclear Cell Isolation and Culture

All reagents were from Sigma-Aldrich (Milan, Italy) unless specified. Lamina propria mononuclear cells (LPMC) were prepared as described elsewhere[3] and used for assessing IL-25R or purifying $CD14^+$ cells by positive MACS separation (Miltenyi Biotec, Bologna, Italy). Cells were resuspended in complete RPMI 1640 medium and cultured with or without IL-25 (10–100 ng/mL; R&D Systems, Inc., Minneapolis, MN) for 30 minutes and then stimulated with lipopolysaccharide (LPS) (100 ng/mL) or peptidoglycan (PGN; 10 μg/mL) for 6–48 hours.

Flow Cytometry

To characterize IL-25R expression in human LPMC and $CD14^+$ cells, the following monoclonal anti-human antibodies were used: CD3 PerCP (1:50, final dilution; Becton Dickinson, Milan, Italy), CD14 FITC (1:50 final dilution, Immunotools, Friesoythe; Germany), isotype control IgGs (Becton Dickinson), IL-25R PE (1:40 final dilution, clone 170220, catalog No. FAB1207P; R&D Systems). Toll-like receptor (TLR)-2 and TLR-4 were evaluated using an anti-human TLR-4 PE (clone HTA125) or TLR-2 (clone TL2.1) PE antibody (both at 1:5 final dilution; eBioscience, San Diego, CA). To assess cell apoptosis, cells were cultured as indicated, and the percentage of annexin V and/or propidium iodide-positive cells was then assessed by flow cytometry according to the manufacturer's instructions (Immunotech, Marseille, France). For mouse experiments, LPMC were stained with CD11b FITC (clone M1/70; BD Pharmigen, Milan, Italy), CD11c PE (clone HL3; BD Pharmigen), F4/80 Alexa Fluor 488 (clone BM8; Biolegend, San Diego CA), and IL-25R APC (clone 152316; R&D Systems).

Real-time Polymerase Chain Reaction

Complementary DNA was amplified using the following conditions: denaturation 1 minute at 95°C, annealing 30 seconds at 58°C for mouse IL-12/p40 and IL-23/p19, and at 62°C for β-actin, followed by 30 seconds of extension at 72°C. Primers sequence was as follows: mouse IL-12p40, FWD: 5′-GGTTTGCCATCGTTTTGCTG-3′ and REV: 5′-GAGGTTCACTGTTTCTCCAG-3′; mouse IL-23p19, FWD: 5′-CTGTAATGCTGCTGTTGCTG-3′ and REV: 5′-CTCTTCATCTCCCTCTTCTC-3′; human IL-12/p40, mouse IL-12/p35, and human IL-25 were evaluated using commercially available TaqMan probes (Applied Biosystems, Foster City, CA). β-actin (FWD: 5′-AAGATGACCCAGATCATGTTTGAGACC-3′ and REV: 5′-AGCCAGTCCAGACGCAGGAT-3′) was used as an internal control.

Experimental Colitis

PGN-colitis was induced in Balb/c mice as previously indicated.[17] For induction of chemically induced colitis, 2.5 mg of 2,4,6-trinitrobenzenesulphonic acid (TNBS) or 4.5 mg of oxazolone in 50% ethanol was administered to mice as previously described.[18] Controls consisted of mice treated with 150 μL of 50% ethanol. Recombinant mouse IL-25 (10 μg/mouse) was injected intraperitoneally 1 hour before the administration of PGN in the preventive model, whereas the cytokine was injected 1 day after the administration of PGN, TNBS, or oxazolone in the therapeutic model. Weight changes were recorded daily, and tissues were collected for histology, protein analysis, and isolation of LPMC. To assess the role of IL-13 in the control of PGN-colitis by IL-25, a neutralizing murine IL-13 or nonrelevant control antibody (250 μg/mouse; R&D Systems) was given 1 hour before IL-25 treatment. At the end of experiments, colons were removed and photographed, and colonic tissues were embedded in paraffin. For PGN-induced colitis, H&E-stained sections were examined for the evidence of colitis, and the degree of inflammation was graded semiquantitatively from 0 to 4 (0, no signs of inflammation; 1, very low level; 2, low level of leukocytic infiltration; 3, high level of leukocyte infiltration, high vascular density, and thickening of the colon wall; 4, transmural infiltrations, loss of goblet cells, ulcerations, high vascular density, and thickening of the colon wall). For TNBS- and oxazolone-induced colitis, the colitis score was assigned as described elsewhere.[18] LPMC were isolated from mice