Interleukin-13 Is the Key Effector Th2 Cytokine in Ulcerative Colitis That Affects Epithelial Tight Junctions, Apoptosis, and Cell Restitution

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Background & Aims: **Ulcerative colitis (UC) is characterized by a Th2 immune response with inflammation and epithelial barrier dysfunction. So far, Th2 cytokines have not been shown to directly influence epithelial barrier function.** ***Methods:*** **Lamina propria mononuclear cells (LPMCs) were stimulated and interleukin (IL)-13 was measured by enzyme-linked immunosorbent assay. Functional IL-13 and IL-4 effects were studied on HT-29/B6 colonic epithelial cells in Ussing chambers and by conductance scanning. Apoptosis was detected by terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling assays. IL-13/IL-4 receptors were analyzed by reverse-transcription polymerase chain reaction and immunofluorescence. Western blotting combined with immunofluorescence was used to detect tight junction proteins. Furthermore, restitution velocity was measured. Finally, mucosal biopsy specimens from patients with UC were compared with cultured cells for these features.** ***Results:*** **LPMCs from patients with UC produced large amounts of IL-13 (985 ± 73 pg/mL), much more than from controls or patients with Crohn's disease. IL-13Rα1 and IL-4Rα receptors were present in HT-29/B6 cells and colonic epithelial cells of control patients and patients with UC. IL-13 had a dose-dependent effect on transepithelial resistance of HT-29/B6 monolayers (reduction to 60% ± 4%), whereas IL-4 had no effect. This was due to an increased number of apoptotic cells (5.6-fold ± 0.9-fold) and an increased expression of the pore-forming tight junction protein claudin-2 to 295% ± 37%, both of which contributed equally. Finally, epithelial restitution velocity decreased from 15.1 ± 0.6 to 10.6 ± 0.5 μm/h after treatment with IL-13. Parallel changes were observed in human samples, with an increase in claudin-2 expression to 956% ± 252%.** ***Conclusions:*** **IL-13 was identified as an important effector cytokine in UC that impairs epithelial barrier function by affecting epithelial apoptosis, tight junctions, and restitution velocity.**

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory bowel diseases (IBDs) characterized by an activated mucosal immune system leading to impaired epithelial barrier function and tissue destruction. Current experimental data suggest that the intestinal flora is an important antigenic stimulus. This has been shown in animal models of IBD, where the gut flora is essential for disease induction, and in humans, because antibiotic treatment or diversion of the fecal stream can ameliorate disease activity.[1]

Whereas an inflammatory response to these antigens from the lumen is suppressed in healthy individuals, a destructive immune response is initiated in patients with IBD. This immune response is mediated by lymphocytes that can either be of a Th1 or a Th2 phenotype. In CD, the inflammation is clearly a Th1 response because it is associated with high levels of interleukin (IL)-12 and interferon (IFN)-γ secretion.[2] These cytokines lead to macrophage and granulocyte activation and thus the release of multiple downstream inflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-6. The result is a transmural (sometimes granulomatous) inflammation that is not primarily centered on epithelial cells, although collateral damage to the epithelium may occur.

Until recently, the inflammation in UC has been difficult to classify using the Th1/Th2 paradigm.

Abbreviations used in this paper: EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; FACS, fluorescence-activator cell sorting; IFN, interferon; IL, interleukin; LDH, lactate dehydrogenase; LPMC, lamina propria mononuclear cell; NF-κB, nuclear factor κB; NKT cell, natural killer T cell; PCR, polymerase chain reaction; P-STAT-6, phosphorylated STAT-6; R^t, transepithelial electrical resistance; TNF, tumor necrosis factor; TUNEL, terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling.

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Whereas in patients with UC the secretion of IFN-γ or IL-12 is not increased and thus the inflammation is clearly not a Th1 response, IL-4 (messenger RNA [mRNA] or protein) is also reduced.[2,3] A breakthrough in our understanding of the disease came initially from the study of oxazolone colitis, a murine model of mucosal inflammation that resembles UC and is caused by IL-13–producing natural killer T cells (NKT cells). Based on these observations, we have recently shown that UC is also associated with increased IL-13 production by NKT cells and that the latter cells manifest reactivity to antigens presented by epithelial cells.[4] These immunologic changes may explain the fact that the inflammation in UC is different from that in CD in that it is a relatively superficial process marked by abnormalities of the epithelium. Ulcers ranging in size from microerosions to large defects disrupt the line of epithelial cells. Abscesses can be found in the base of the crypts. Already early in the disease process, the barrier function of the mucosa is severely impaired.[5] This arises from widespread apoptosis of epithelial cells and a decreased complexity of the tight junctions between epithelial cells causing increased paracellular permeability.[6]

Here we report findings indicating that most of the functional defects of the mucosa can in fact be traced to direct effects of IL-13 on epithelial cells. This includes epithelial tight junction alterations and stimulation of epithelial apoptosis together with epithelial restitution arrest. Thus, IL-13 emerges as a key effector cytokine in UC acting adversely on various aspects of epithelial cell function that ultimately lead to the severe destructive inflammation seen in patients with UC.

Materials and Methods

Lamina Propria Mononuclear Cell Cytokine Production

Lamina propria mononuclear cells (LPMCs) were isolated from surgical specimens of patients undergoing colectomy as described previously.[2] Six patients had chronic active UC, and 5 patients had CD; 4 patients without intestinal inflammation with colonic adenocarcinoma served as noninflammatory controls. The institutional review boards approved the collection of surgical specimens. Because repetitive culture of cells from the same surgical specimen showed almost identical cytokine production, we used each specimen only once for LPMC stimulation experiments and each measurement was obtained from another specimen. Freshly isolated LPMCs were cultured and stimulated in vitro with soluble antibodies to CD2 and CD28. IL-13, IL-4, and IFN-γ were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN; Pierce Chemical Co, Rockford, IL; and BD PharMingen, New York, NY) in culture supernatants collected 48 hours after stimulation as described previously.[4]

Detection of IL-13 Receptors and Claudin-2 mRNA

IL-13Rα1, IL-13Rα2, and IL-4Rα were detected by reverse-transcription polymerase chain reaction (PCR) and immunofluorescence. Claudin-2 mRNA was quantified by real-time PCR. RNA was isolated from IL-13–treated (48 hours; 10 ng/mL) or control cultures of HT-29/B6 cells with RNAzol B (Wak-Chemie Medical GmbH, Steinbach, Germany) according to the manufacturer's protocol. After reverse transcription of 1.5 μg total RNA for real-time PCR or 2 μg total RNA for receptor PCR (Omniscript RT Kit; Qiagen, Hilden, Germany) (60 minutes at 37°C, 5 minutes at 93°C), IL-13 or IL-4 receptor complementary DNA (cDNA) was amplified (35 cycles of 45 seconds at 95°C, 60 seconds at 60°C, and 60 seconds at 68°C) with the following primer pairs: hIL13Rα1FOR ggagccagctcaatttgtag, hIL13Rα1REV cacacgggaagttaaaggca, hIL13Rα2FOR ggagagaggcaatatcaagg, hIL13Rα2REV ggccatgactggaaactgt, hIL4RFOR gacctggagcaacccgtatc, and hIL4RREV catagcacaacaggcagacg. The amplified products were verified by agarose gel electrophoresis and showed single bands of predicted sizes for each sample and no products in negative controls. In biopsy samples from patients with UC or noninflammatory controls, IL13Rα1 and IL4Rα (both with antibodies from R&D Systems, Minneapolis, MN) were detected by immunofluorescence according to the protocol listed in the following text. cDNA prepared from untreated or IL-13–treated HT-29/B6 cells as described previously was amplified with claudin-2 specific primers: CLDN2F gaatcccgagccaaagacagagtg and CLDN2B tcaggagaacagggaagaaataa. Quantitative LightCycler-PCR was performed using the FastStart DNA Master SYBR Green I Kit according to the manufacturer's instructions (Roche, Mannheim, Germany). The final $MgCl_2$ concentration was 3 mmol/L. Each sample contained 3 μL cDNA preparation. The reaction mixture was denatured for 10 minutes at 95°C and subjected to 40 cycles in a 3-step PCR (95°C for 15 seconds, 60°C for 5 seconds, and 72°C for 10 seconds). Detection of fluorescence occurred at the end of the 72°C elongation step. Specificity of PCR products was verified by melting curve analysis subsequent to the amplification. Amplification, data acquisition, and analysis were performed by LightCycler (Roche). Standardization was performed with a standard dilution of a pCR2.1-TOPO vector construct (Invitrogen, Karlsruhe, Germany) containing the 199–base pair amplicon generated by the primer pair CLDN2F and CLDN2B. Claudin-2 mRNA copies were expressed per nanograms total RNA.

Cell Culture of HT-29/B6 Cells

HT-29/B6 cells represent a subclone of the human colorectal cancer cell line HT-29, which grow as highly differentiated polarized epithelial monolayers.[7] The cells were routinely cultured in 25-cm^2 culture flasks (Nunc, Wiesbaden, Germany). The culture medium contained RPMI 1640, 2%