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Original Contribution

Immunomodulatory cytokines suppress epithelial nitric oxide production in inflammatory bowel disease by acting on mononuclear cells

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Abstract

Inducible nitric oxide synthase (iNOS) activity in colonic epithelial HT-29 cells is modulated by the T-cell-derived cytokines IL-4 and IL-13, but is not affected by IL-10 despite its effect in models of colitis. We studied the effects of these cytokines on nitric oxide (NO) production by colonic tissue. IL-10 and IL-4 but not IL-13 suppressed the NO production and iNOS expression by inflamed tissue and cytokine-stimulated noninflamed tissue from patients with ulcerative colitis, whereas the three cytokines suppressed NO production in cytokine-stimulated biopsies from controls. To examine why colonic biopsies and HT-29 cells respond differently to immunomodulatory cytokines, a coculture of mixed mononuclear monocytes (MMC) and HT-29 cells was studied. Treatment of HT-29 cells with conditioned medium from IFN-γ/LPS-stimulated MMC produced significant amounts of NO, which suggested the presence of an MMC-derived soluble factor modifying epithelial NO production. Pretreatment of IFN-γ/LPS-stimulated MMC with IL-10 and IL-4 but not IL-13 suppressed NO production by HT-29 cells. Interestingly, pretreatment of HT-29 cells with IL-1 receptor antagonist suppressed the IFN-γ/LPS-stimulated MMC-induced NO production. These results suggest that immunomodulatory cytokines might exert an inhibitory effect on NO up-regulation by colonic epithelium via the inhibition of MMC-derived soluble mediators, such as IL-1.
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There has been much interest recently concerning the role of the immunomodulatory cytokines interleukin-4 (IL-4), IL-13, and IL-10 in inflammatory bowel disease (IBD) and their potential use as therapeutic agents. Most work, now at the clinical trial stage, has been done on IL-10. We have previously shown that nitric oxide (NO) produced by the colonic epithelial cell is important in the pathophysiology of IBD and that IL-4 and IL-13 are capable of regulating its production [1].

IL-4 is primarily produced by T cells and has a wide range of activities such as the augmentation of MHC class II antigen expression on B cells, stimulation of mast cells and T cells, modulation of NK cells, and stimulation of hemopoietic cells. Despite the variety of effects on several different immune cells and functions relevant to the modulation of immunity and the possible down-regulation of the immune response, little is known about the role of IL-4 in IBD. IL-4 has been shown to modulate the proliferation and cytotoxicity of lamina propria mononuclear cells. This effect seems to be differentially expressed in different forms of IBD, with IL-4 inhibiting lymphokine-activated killer cells in ulcerative colitis and in controls but not in Crohn disease patients [2,3].

Interleukin-10 is produced by a variety of cells, including Th2 cells, mast cells, and cells of the macrophage lineage. It

Abbreviations: NO, nitric oxide; IBD, inflammatory bowel disease; iNOS, inducible nitric oxide synthase; MMC, mixed mononuclear cells; CM, conditioned medium; IL-1ra, interleukin 1 receptor antagonist.

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acts as a suppressor of cytokine expression through the deactivation or inhibition of monocytes and macrophages [4]. A role in the pathogenesis of IBD is suggested by the discovery that mice deficient in the IL-10 gene spontaneously develop an enterocolitis [5]. Inflamed gastrointestinal mucosa in humans contains very high levels of IL-10 mRNA [6,7]. Patients with ulcerative colitis (UC) or Crohn disease have high circulating levels of IL-10 [8]. This has led to the suggestion that IL-10 may act as an anti-inflammatory agent in IBD and thus studies have been performed using IL-10 in the treatment of colitis both in animal studies [9,10] and in humans [11–13]. These studies have shown that in IBD the effects of IL-10 are weak, but would suggest that this is an area likely to grow in importance.

IL-13 has multiple documented effects such as potent suppression of cytokine and chemokine expression by activated monocytes and macrophages [14,15], induction of IL-1 receptor antagonist, and modulation of expression of cell surface proteins such as class II MHC antigens [16,17]. The results on IL-13 production in IBD patients are contradictory so far [18]. The inhibitory effect of IL-13 on the production of the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and IL-6 in differentiated macrophages was diminished in IBD patients and the anti-inflammatory activity of IL-13 was partially reduced in patients with active IBD [3].

Nitric oxide is produced throughout the gastrointestinal tract. It is believed to be important in both physiological and pathological events depending on the quantity of NO produced and when it is produced in relation to the time of the injury [7]. The colonic epithelial cell has been previously identified as the cellular source of NO via expression of iNOS in the colonic mucosa in IBD [19]. Studies using immunostaining and in situ hybridization have demonstrated high expression of iNOS localized to the surface epithelium and crypts in the colonic mucosa from patients with UC [1,19]. We have demonstrated previously that the colonic epithelial cell line HT-29 produces large quantities of NO by induction of the inducible isoform with increased expression of mRNA for iNOS when stimulated with a "cocktail" of proinflammatory cytokines (interleukin-1α, TNF-α, and interferon-γ). This production can be successfully inhibited by pretreatment with either IL-4 or IL-13 but not IL-10 [1].

The observation that proinflammatory cytokines induce NO production and iNOS activity in colonic epithelial cells, whereas Th2 and other immune-cell-derived mediators regulate this process, suggests an implication of colonic epithelial cells in NO overproduction and cell communication during intestinal inflammation. Excess NO produced by the inducible enzyme may theoretically exacerbate the clinicopathological features of UC by direct cytotoxicity, activation of neutrophils [20], vasodilatation, reduced smooth muscle tone [21], increased production of nitrosamines [22], and interaction with superoxide to form the highly toxic peroxynitrite radical [19]. However, it is not fully defined yet how the overall modulation of NO production in the colonic wall can affect IBD progression.

This study is a series of experiments to examine further the controlling mechanisms of NO production in the colonic epithelial cell and specifically how the group of anti-inflammatory cytokines IL-4, IL-10, and IL-13 affects this production. IL-10 does seem to have some clinical effects in the treatment of IBD yet does not affect NO production in the cell line model described previously [1]. Thus we set out to look at how these three cytokines affect iNOS-derived NO in a tissue and cell culture model of inflammation and in specimens from patients with active newly diagnosed ulcerative colitis.

Materials and methods

Materials

Recombinant human (rh) IL-1α and TNF-α were gifts from Glaxo (Greenford, Middlesex, UK) and Bayer (Slough, Berkshire, UK), respectively. Recombinant human interferon-γ (IFN-γ) and rhIL-10 were purchased from Boehringer Mannheim (Lewis, Sussex, UK). rhIL-13 was purified from cultured supernatants of stable transfected CHO cells and was generously provided by Dr. A. Minty (Sanofi Recherche, Labege, France). rhIL-4 was purchased from Genzyme. Oligonucleotide primers were synthesized by PE Applied Biosystems (Warrington, UK). 2,3-Diaminonaphthalene (DAN) was purchased from Lancaster Synthesis Ltd. (Newgate, UK). Sodium nitrite, lipopolysaccharide (LPS) and standard reagents were purchased from Sigma (Poole, UK). Cell culture reagents and fetal bovine serum (FBS) were from Gibco BRL (Paisley, UK).

Cell cultures

The HT-29 cell line was purchased from the European Collection of Animal Cell Cultures. Cell cultures were maintained in McCoy's 5A medium supplemented by 10% FBS, penicillin–streptomycin (10 U/ml and 10 mg/ml), and Fungizone (0.5 μg/ml) and incubated at 37°C in an atmosphere of 5% CO_2 until confluent. Confluent cell cultures were treated with the appropriate concentrations of stimuli, as previously described [23].

Colonic biopsies

Multiple colonic biopsies were taken from patients who underwent colonoscopy at the Royal United Hospital (Bath, UK). The study included: (1) biopsies from inflamed mucosa of patients with a new clinical diagnosis of ulcerative colitis who had yet to start on any treatment ($n = 17$) (8 men and 9 women, mean age 44.9 years, range 16–81), (2) biopsies from histologically normal mucosa of the proximal colon

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