Degeneration of the Pericryptal Myofibroblast Sheath by Proinflammatory Cytokines in Inflammatory Bowel Diseases

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Background & Aims: **Inflammatory bowel diseases (IBDs) are characterized by remodeling of the intestinal mucosa, which is associated with excessive cytokine release. Previous studies have shown that the epithelium in the crypt region of the mucosa in patients with Crohn's disease is susceptible to proinflammatory cytokines. We investigated whether the subepithelial myofibroblasts in this region were affected by these inflammatory conditions.** ***Methods:*** **Immunofluorescence and immunohistochemistry were performed on inflamed and uninflamed specimens from patients with IBD to detect α-smooth muscle actin (αSMA), desmin, and tenascin-C. The effects of the proinflammatory cytokines interleukin-1β, tumor necrosis factor-α, and interferon-γ were analyzed in human intestinal myofibroblast cultures by immunoblotting and apoptosis assays.** ***Results:*** **Immunofluorescence analysis revealed decreased levels of the extracellular matrix molecule tenascin-C in pericryptal sheaths and αSMA in the immediate vicinity of the crypts in the inflamed specimens, indicating that the myofibroblast pericryptal sheath is affected by proinflammatory cytokines. Although individual cytokines did not affect myofibroblast proliferation or survival, cytokine combinations triggered caspase-dependent apoptosis. αSMA levels were reduced significantly in cells exposed to cytokines, either alone or in combination, suggesting dedifferentiation of myofibroblasts. Proinflammatory cytokines did not affect tenascin-C expression, suggesting that the decrease observed in the inflamed mucosa resulted from myofibroblast apoptosis.** ***Conclusions:*** **The subepithelial myofibroblasts of the epithelial sheath are disrupted in the intestinal mucosa of patients with IBD. A loss of myofibroblasts appears to result from the susceptibility of these cells to proinflammatory cytokines.**

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. As with other inflammatory bowel diseases (IBDs), CD is a multifactorial disorder whose pathogenesis involves genetic predisposition in combination with yet incompletely defined host and environmental factors.[1–3] The cascade of events leading to the development of CD remains poorly understood. Although many lines of evidence suggest the involvement of a primary immunologic defect,[4] the recent discovery of a number of susceptibility genes[5] expressed in various cell types and the well-documented impairment of the intestinal barrier[6] argue for the direct implication of nonhematopoietic components. It therefore appears more and more evident that the intestinal inflammatory response relies on a complex interplay between immune and nonprofessional immune cell interactions.[2,7]

Involvement of nonprofessional immune cell types in the pathogenesis of IBDs has been well exemplified in epithelial cells. After the initial observations reporting morphologic and functional alterations of epithelial cell integrity in IBDs, evidence has been provided that these changes are mediated primarily by cytokines released from adjacent inflammatory cells as well as from the epithelial cells themselves.[7–9] Epithelial cells have been shown to express a wide repertoire of functional receptors for cytokines such as tumor necrosis factor-α (TNFα), interferon-γ (IFNγ), transforming growth factor-β (TGFβ), and various interleukins. The action of these cytokines on intestinal epithelial cell models has been characterized as affecting several key cell functions, including proliferation, paracellular permeability and claudin expression, cytokine and extracellular matrix (ECM) molecule expression, as well as cell survival.[8–11]

The myofibroblast is another nonprofessional immune mucosal cell type for which a growing body of evidence supports a participation in the pathogenesis of IBD.[2,7] Myofibroblasts represent an intermediate cell type between smooth muscle cells and fibroblasts[12] that are

Abbreviations used in this paper: αSMA, α-smooth muscle actin; CMF, colonic myofibroblasts; ECM, extracellular matrix; HIM, human intestinal myofibroblast; I-CD, inflamed Crohn's disease; IFNγ, interferon; IL, interleukin; TGFβ, transforming growth factor; Tn-C, tenascin-C; TNFα, tumor necrosis factor; U-CD, uninflamed Crohn's disease.

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characterized by smooth muscle actin (αSMA) and vimentin expression.[13] Although there is evidence for the involvement of intramucosal myofibroblasts in CD,[14] it is the intestinal subepithelial myofibroblasts present immediately subjacent to the epithelial basement membrane[15] in the form of a pericryptal sheath that have been the primary focus of attention in the pathogenesis of IBD. Their proximity to the basal surface of epithelial cells makes them potential targets for the bacteria and/or bacterial products deposited in the subepithelial compartment in the presence of disruption in the epithelial barrier. Indeed, stimulated intestinal myofibroblasts have been shown to express various cytokines and chemokines[7,15,16] as well as ECM molecules such as tenascin-C.[17] Moreover, it recently was suggested that intestinal subepithelial myofibroblasts participate in the innate immune response.[18,19] Furthermore, as central components of epithelial-mesenchymal interactions in the gut,[15,20] subepithelial myofibroblasts have been characterized as modulating both basic and healing epithelial cell functions such as migration, proliferation, differentiation, and survival through the following mechanisms: (1) the secretion of various paracrine factors such as the Wnts, Bmps, and TGFβ, targeting epithelial cells[15,20]; (2) the release of various proteinases[21,22]; and (3) the production of a number of ECM molecules that contribute to the epithelial basement membrane.[17,23,24]

However, despite their potential involvement in the immune response, little is known about the potential role of intestinal subepithelial myofibroblasts in the course of the chronic inflammatory process that characterizes IBD. In this work, using specific cell markers, we have analyzed the behavior of intestinal subepithelial myofibroblasts and alterations in the organization of the pericryptal sheath in paired inflamed and uninflamed specimens of CD. We also investigated the effect of various cytokines on cultures of intestinal myofibroblasts to examine myofibroblastic cell proliferation, differentiation, and survival in a proinflammatory-like context.

Materials and Methods

Tissues

The CD ileal specimens used (n = 11) were obtained from therapeutic resections of consenting patients, as characterized previously.[25] Control specimens (n = 6) were obtained from the nondiseased parts (at least 10-cm distant from lesions) of resected ileum for pathologies other than CD (bowel obstruction, primary lymphoma, or other tumors). Another 13 specimens obtained from colon affected with various pathologies (3 diverticulitis including normal adjacent mucosa used as control; 5 acute inflammatory colitis: 3 infectious colitis and 2 noninfectious colitis; and 5 IBD: 3 CD, 2 ulcerative colitis) were obtained from the Department of Pathology, Université de Sherbrooke from consenting patients. The procedures were in accordance with a protocol approved by the Institutional Human Review Board for the use of human material. The preparation and embedding of tissues was performed as previously described for cryosectioning[25,26] and according to standard pathologic procedures for paraffin sections.

Indirect Immunofluorescence and Immunohistochemistry

Cryosections 2–3 μm thick were fixed in paraformaldehyde as described.[25,26] Tenascin-C (Tn-C) was detected with the polyclonal anti–Tn-C antibody (Chemicon, Temecula, CA). αSMA was detected with the monoclonal 1A4 antibody (Sigma–Aldrich, Oakville, Ontario, Canada). Desmin was detected with the polyclonal 2P3M (Biomeda, Foster City, CA). The secondary antibodies used were fluorescein isothiocyanate– or rhodamine-conjugated anti-rabbit or anti-mouse immunoglobulin G (Roche Diagnostics, Laval, Quebec, Canada). For paraffin sections, αSMA and desmin were detected with the monoclonal 1A4 (Dako Canada, Mississauga, Ontario, Canada) and DE-R-11 (Novo Castra, Leica Canada, Richmond Hill, Ontario, Canada) antibodies.

RNA Extraction and Reverse-Transcriptase Polymerase Chain Reaction

Human intestinal myofibroblast (HIM) cells were seeded in 24-well plates (Sarstedt, Montréal, Quebec, Canada) at a density of 1×10^5 cells/well under serum-free conditions and left untreated or treated with TNFα/IFNγ (10 ng/mL each) or TGFβ (2.5 ng/mL) for 72 hours. Total RNAs from 6 series of paired inflamed and uninflamed CD specimens[25] were prepared according to Clontech's protocol (Clontech, Palo Alto, CA). The reverse-transcriptase Omniscript (Qiagen, Mississauga, Ontario, Canada) and 1 μmol/L of oligo$(dT)_{12-18}$ (GE Healthcare, Baie d'Urfé, Québec, Canada) were added to 2 μg of total RNA, according to the manufacturer's instructions. Primers for S14 amplification have been described elsewhere.[27] For αSMA, we used the sense primer 5′-TTCAATGTCCCAGCCATGA-3′ and the antisense primer 5′-GAAGGAATAGCCACGCTCAG-3′, and for Tn-C amplification we used the forward 5′-GCATCCACTGCCATGGGCT-3′ and reverse 5′-CTCCACGCCAGGTATGAGTT-3′ primers. For quantitative evaluation of transcript levels, real-time experiments were performed using an Mx3000P (Stratagene, Cedar Creek, TX) as previously described.[27] Relative quantification was performed with the same S14 primers as described earlier.

Myofibroblasts

HIM cells were obtained from the ileum of 16- to 18-week-old fetuses using the outgrow explant method described previously.[28] The cells were grown in OPTI-MEM (Invitrogen, Burlington, Ontario, Canada) sup-