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Proinflammatory cytokines induce crosstalk between colonic epithelial cells and subepithelial myofibroblasts: Implication in intestinal fibrosis ☆

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Abstract

Background and aims: Colonic epithelial cells and adjacent subepithelial myofibroblasts are important counterparts in the pathogenesis of intestinal inflammation and fibrosis. We investigated the possible crosstalk between them, whilst focusing on the mucosal inflammation pathways that potentially trigger intestinal fibrosis.
Methods: We studied the effects of proinflammatory cytokines (IL-1α, TNF-α, IFN-γ) on human colonic epithelial cell lines and the effects of epithelial cell-conditioned media on primary human colonic subepithelial myofibroblasts isolated from normal controls or patients with inflammatory Crohn's disease along with the corresponding 18CO cell line. Readouts included production of TGF-β and TIMP-1, total collagen synthesis, matrix metalloproteinases MMP-2 and MMP-9 and myofibroblast migration/mobility.

Abbreviations α-SMA, alpha-smooth muscle actin; CD, Crohn's disease; CTGF, connective tissue growth factor; ECC, epithelial cell-conditioned; ELISA, enzyme-linked immunosorbent assay; ECM, extracellular matrix; ET, endothelin; ETR, endothelin receptor; HGF, hepatocyte growth factor; IBD, inflammatory bowel disease; IFN-γ, interferon-gamma; IL-1α, interleukin-1alpha; MMP, matrix metalloproteinase; SEMF, subepithelial myofibroblast; TF, tissue factor; TGF-β, transforming growth factor-beta; TIMP-1, tissue inhibitor of metalloproteinases-1; TNF-α, tumour necrosis factor alpha.

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Results: Proinflammatory cytokines upregulated TGF-β and TIMP-1 in colonic epithelial cells. Conditioned medium from these epithelial cell cultures induced production of MMP-9 and collagen and inhibited the migration/mobility of subepithelial myofibroblasts. MMP-9 production depended on endothelin receptor A signalling on responding myofibroblasts. Collagen up-regulation was independent of TGF-β, CTGF, TF and endothelin. Subepithelial myofibroblasts isolated from Crohn's disease patients had similar responses to those isolated from normal controls, with the exception of higher basal collagen production.
Conclusions: Our study indicates that colonic epithelial cells may respond to an inflammatory *milieu* by inducing myofibroblast functions similar to those observed during intestinal fibrosis.

1. Introduction

Intestinal subepithelial myofibroblasts (SEMFs) are alpha-smooth muscle actin (α-SMA)-positive mesenchymal cells located at the interface between the intestinal epithelium and lamina propria. Connective tissue fibrils form a connective tissue barrier called the basal lamina, through which SEMFs and the epithelium can extend interacting pseudopods.[1,2] The epithelial–mesenchymal cell interaction may play an important role in the process of intestinal extracellular matrix (ECM) remodelling and inflammation associated fibrosis. Mucosa overlying Crohn's disease (CD) strictures overexpress profibrotic transforming growth factor-beta (TGF-β) transcripts and tissue inhibitor of metalloproteinases-1 (TIMP-1),[3,4] whilst resident mesenchymal cells overproduce collagens I and III.[5–8] Matrix metalloproteinases (MMPs), including the gelatinases MMP-2 and MMP-9, have also been implicated in ECM remodelling and ulceration in inflammatory bowel disease (IBD).[9–12]

Epithelial cells have been proposed to participate in the control of ECM remodelling in the skin and lungs. In the skin, keratinocytes are partially responsible for the induction of α-SMA in fibroblasts via TGF-β and endothelin-1 (ET-1).[13] Interactions between keratinocytes and the underlying fibroblasts also seem to modulate the levels of MMP-2 and MMP-9.[14] In the lungs, mechanically stressed epithelial cells induce the incorporation of proline into matrix proteins (mostly collagen) by unstressed normal human lung fibroblasts via pathways that involve ET and TGF-β2.[15]

Our study explored possible interactions between epithelial cells and adjacent SEMFs in the gut. We studied the effects of proinflammatory cytokines on the production of profibrotic mediators by epithelial cells, and the effects of proinflammatory cytokines and epithelial cell conditioned medium on MMP activity, collagen production and mobility/migration of colonic subepithelial myofibroblasts isolated from normal controls and CD patients.

2. Materials and methods

2.1. Materials

For cell culture treatments, human interleukin-1alpha (IL-1α), tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ) and active recombinant transforming growth factor-β1 (TGF-β1) were purchased from R&D Systems (Abingdon, UK). Mouse monoclonal anti-human antibody was also purchased from R&D Systems (Abingdon, UK) to neutralise all TGF-β isoforms (anti-pan-TGF-β). Neutralising rabbit anti-human connective tissue growth factor (CTGF) polyclonal antibody was purchased from Acris Antibodies GmbH (Hiddenhausen, Germany) and neutralising anti-human monoclonal tissue factor (TF) antibody was purchased from American Diagnostica (Stamford, CT). Human anti-thrombin-III protein (Kybernin® P) was purchased from ZLB Behring (Hattersheim am Main, Germany). We used azepane-1-carbonyl-Leu-D-Trp(For)-D-Trp-OH (BQ-610) and N-cis-2,6-dimethylpiperidinocarbonyl-β-tBu-Ala-D-Trp(1-methoxycarbonyl)-D-Nle-OH (BQ-788) as selective synthetic antagonists for endothelin receptors (ETR) A and B, respectively; these chemicals were purchased from Bachem (Weil am Rhein, Germany).[16] Goat anti-human polyclonal anti-CTGF IgG and goat anti-human monoclonal IgG1 isotype control antibodies used for immunocytochemistry studies were products of Santa Cruz Biotechnologies (Santa Cruz, CA) and DAKO (Carpinteria, CA), respectively.

2.2. Patients

Colonic tissue was obtained by endoscopic biopsy from patients with CD and normal controls undergoing colonoscopy at the Gastroenterology Department, University Hospital of Heraklion, Greece. Colonic biopsies were obtained from 10 control patients, who underwent diagnostic colonoscopy for reasons other than IBD, (e.g. abdominal pain, screening colonoscopy) and where the examination and histology were found to be normal. Biopsies of inflamed colonic mucosa were obtained from three patients with CD ileocolitis. All patients were first diagnosed, with CD proven by colonoscopy and histological examination, they had a Crohn's disease Activity Index >150 and they did not receive any treatment prior to examination. The local Research Ethics Committee has granted approval for this study, and patients gave their informed written consent prior to participation in the study.

2.3. Colonic subepithelial myofibroblast isolation and culture

Studies were performed on subepithelial myofibroblasts (SEMFs; passages 3–8) isolated from colonic tissue. Tissue was obtained by endoscopic biopsy of apparently normal mucosa from patients undergoing screening colonoscopy or inflamed colonic mucosa from the three patients with CD