Journal of Crohn's and Colitis (2014) xx, xxx–xxx

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Effect of interleukin-17 on gene expression profile of fibroblasts from Crohn's disease patients

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Received 5 December 2013; received in revised form 14 February 2014; accepted 14 February 2014

KEYWORDS
Inflammatory bowel disease;
Cytokines;
Chemokines;
Fibroblasts;
Interleukin 17;
Inflammation

Abstract

Background and aim: The expression of interleukin (IL)-17 is upregulated in inflammatory bowel disease (IBD). Since fibroblasts are known to be responsive to IL-17, they may play a role in the modulation of inflammatory responses in IBD. Here, the effects of IL-17 on ileum and colon fibroblasts from Crohn's disease (CD) and ulcerative colitis (UC) patients are investigated, as compared to controls.
Methods: Fibroblasts were isolated from surgical specimens taken from the tissue of 21 CD patients, 5 UC patients, and 14 patients undergoing surgery for colorectal carcinoma (control). The fibroblasts were cultured with and without IL-17. We performed mRNA microarray analysis on cultured fibroblasts, isolated from three CD samples and three control samples. Based on these results, the expression of IL-17 induced genes was validated in a larger selection of samples using qRT-PCR and ELISA.
Results: The mRNA microarray showed that IL-17 induced the expression levels of various genes in fibroblasts of CD patients and controls, among which *NFKBIZ*, *CXCL1*, and *CXCL6* demonstrated the most prominent response. qRT-PCR validated that IL-17 induced the expression of *NFKBIZ* significantly ($p = 0.028$) in intestinal fibroblasts of CD patients. By performing an ELISA, we also discovered that, following IL-17 stimulation, CXCL1 levels were significantly increased in fibroblasts from CD patients ($p = 0.048$). IL-17 also stimulated secretion of CXCL6 in fibroblasts from UC patients ($p = 0.053$).
Conclusion: The enhanced expression of IL-17 that is observed in patients with Crohn's disease could act on intestinal fibroblasts to induce expression of transcription factor *NFKBIZ* and proinflammatory chemokine CXCL1. This can have consequences for fibroblast activity and neutrophil chemotaxis.

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http://dx.doi.org/10.1016/j.crohns.2014.02.009
1873-9946/

Please cite this article as: Kerami Z, et al, Effect of interleukin-17 on gene expression profile of fibroblasts from Crohn's disease patients, *J Crohns Colitis* (2014), http://dx.doi.org/10.1016/j.crohns.2014.02.009

1. Introduction

Numerous cell types constitute inflammatory bowel disease (IBD) pathogenesis through complex mutual cross talks that are mediated by both the immune and non-immune systems.[1] Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of IBD. Although its etiology remains poorly understood, several genetic, immune, and environmental factors have been shown to be involved in the pathogenesis of IBD.[2,3]

One of the common consequences of this chronic progressive disease is intestinal fibrosis, which can occur in both forms of IBD. In UC, fibrosis is restricted to the mucosal and submucosal layers, and manifests itself as a shortening or stiffening of the colon, while in CD it can involve the entire intestinal wall and can cause thickening of the bowel wall and formation of strictures.[3–6] Despite the great success of new anti-inflammatory therapies in IBD, there are no effective therapeutic approaches to also treat intestinal fibrosis.[6]

Fibroblasts, which are cells of mesenchymal origin, are considered to be the main effector cells in the pathophysiology of gastrointestinal fibrosis.[3]

Fibroblasts of inflamed intestinal mucosa are exposed to a microenvironment with different cell types and biological mediators.[3] In response to proinflammatory stimuli, fibroblasts produce matrix metalloproteinases (MMPs), collagen, and fibrogenic factors.[7] Several cytokines have been shown to affect intestinal fibroblast gene expression; however, it is still unclear if and how these contribute to the formation of intestinal fibrosis.[8]

One of the cytokines that might play a role in fibrogenesis is interleukin-17 (IL-17).[9,10] This is a proinflammatory cytokine of the IL-17 family of six related cytokines (IL-17A to IL-17F).[11] IL-17 is predominantly secreted by T lymphocytes; namely the T helper 1 (Th1), and Th17 subsets of $CD4^+$ T cells, $CD8^+$ T cells, and $\Upsilon\delta$ T cells. Other cells, such as NkT cells, LTi-like cells, macrophages, neutrophils, and Paneth cells, can also actively secrete IL-17.[9] IL-17 has been considered as a potent mediator of inflammatory responses in various tissues and induces several genes associated with inflammation, including IL-8, IL-6, leukemia inhibitory factor (LIF), intercellular adhesion molecule (ICAM)-1, and granulocyte/macrophage-colony stimulating factor (GM-CSF).[12] Correspondingly, IL-17 induces CXCL1, CXCL2, CXCL5, and CXCL8 in human epithelial cells.[11] In intestinal subepithelial myofibroblasts, IL-17 stimulates secretion of IL-6, IL-8, and MCP-1 via NF-κB and MAP kinase activation.[8]

The effect of IL-17 on its target cells is mediated by five members of the IL-17 receptor (IL-17R) family (IL-17RA to IL-17RE).[10] The IL-17RA is widely distributed in various cell types, such as fibroblasts, epithelial cells, B and T lymphocytes, endothelial cells, macrophages, and keratinocytes.[9] The ligation of IL-17R, depending on the nature of the cells, initiates signaling pathways to induce a wide variety of molecules, ranging from cytokines (IL-6, G-CSF, GM-CSF) to chemokines (CCL2, CCL7, CCL20, CXCL1, CXCL5), and matrix metalloproteinases (MMP1, MMP3, MMP9, MMP12, and MMP13).[13]

IL-17 has been correlated to a growing number of autoimmune and inflammatory diseases such as rheumatoid arthritis, psoriasis, asthma, and IBD.[12,14,15] Patients with IBD display an elevated expression of IL-17 mRNA and intercellular protein in the intestinal mucosa. Expression of IL-17 is detectable in the serum of patients with active exacerbations of IBD.[12] The interaction between IL-17 and subepithelial myofibroblasts is of possible significance in the pathophysiology of gut inflammation.

Next to the pro-inflammatory effects of IL-17, some studies report that IL-17 also induces anti-inflammatory responses in various tissues. It has been shown that IL-17 blocks TNF-α-induced RANTES and IP-10 secretion, which are potent chemoattractants and activators of T cells and monocytes–macrophages. By this action IL-17 can play a role in downregulation of T-cell and monocyte–macrophage-mediating immune responses. Finally, IL-17 might decrease mucosal barrier function via downregulation of both claudin expression and mucin secretion.[16]

There is no doubt about the contribution of IL-17 in IBD; however, the precise role of IL-17 in the pathophysiology of IBD remains unclear. In this study, we investigated the potential role of IL-17 in the induction of inflammatory responses in fibroblasts of CD patients, with a focus on the induction of proinflammatory cytokines and chemokines. To do so, we designed a comprehensive microarray study and validated results using qPCR and ELISA.

2. Materials and methods

2.1. Patient material

Ileum and colon tissue samples (Table 1) were anonymously obtained as residual material from surgical procedures according to the ethical guidelines of the Academic Medical Center (AMC), Amsterdam, The Netherlands.

The experimental group consists of intestinal tissue samples from IBD patients. In accordance with the guidelines of our pathology department, a control group may be set up using colectomy samples of colorectal carcinoma patients. Non-inflamed, macroscopically normal looking tissue has been taken from at least 10 cm away from the tumor.

2.2. Isolation and culture of human fibroblasts from resection specimen

The tissue is prepared following an adapted protocol. First, the mucosa from surgical specimens was cut into small pieces and washed in wash buffer with calcium- and magnesium-free Hanks' balanced salt solution (HBSS; Gibco, Scotland, UK) supplemented with 15 mM HEPES (15 mM, Lonza, Walkersville,

Table 1 Experimental characteristics of the IBD patients.

Characteristics	CD	UC	Total
Numbers	21	5	26
Diseased areas	6		6
Non-diseased areas	7		7
Not defined	8	5	13
Intestinal locations			
Ileum	16		16
Colon	4	2	6
Rectum	1	3	4

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