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Chemokine and cytokine levels in inflammatory bowel disease patients

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ABSTRACT

Crohn's disease (CD) and ulcerative colitis (UC), two forms of inflammatory bowel disease (IBD), are chronic, relapsing, and tissue destructive lesions that are accompanied by the uncontrolled activation of effector immune cells in the mucosa. Recent estimates indicate that there are 1.3 million annual cases of IBD in the United States, 50% of which consists of CD and 50% of UC. Chemokines and cytokines play a pivotal role in the regulation of mucosal inflammation by promoting leukocyte migration to sites of inflammation ultimately leading to tissue damage and destruction. In recent years, experimental studies in rodents have led to a better understanding of the role played by these inflammatory mediators in the development and progression of colitis. However, the clinical literature on IBD remains limited. Therefore, the aim of this study was to evaluate systemic concentrations of key chemokines and cytokines in forty-two IBD patients with a range of disease activity compared to levels found in ten healthy donors. We found a significant increase in an array of chemokines including macrophage migration factor (MIF), CCL25, CCL23, CXCL5, CXCL13, CXCL10, CXCL11, MCP1, and CCL21 in IBD patients as compared to normal healthy donors ($P < 0.05$). Further, we also report increases in the inflammatory cytokines IL-16, IFN-γ, IL-1β and TNF-α in IBD patients when compared to healthy donors ($P < 0.05$). These data clearly indicate an increase in circulating levels of specific chemokines and cytokines that are known to modulate systemic level through immune cells results in affecting local intestinal inflammation and tissue damage in IBD patients. Blockade of these inflammatory mediators should be explored as a mechanism to alleviate or even reverse symptoms of IBD.

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1. Introduction

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), can be classified as an inflamed state of the gastrointestinal tract that is not caused by infection or cancer. IBD affects millions of people globally [1] and the prevalence is increasing annually. The precise mechanism for the development and progression of IBD remains unclear, but accumulating evidence suggests that the pathology is driven by the advancement of immunological lesions that are accompanied by a prominent infiltrate of cells including T lymphocytes, macrophages, neutrophils, and plasma cells [2]. Based on several recent studies in rodents, an imbalance in T helper cells [3–5], IFN-γ overproduction by lamina propria (LP)-macrophages and -T cells [6,7], and domination of Th1 cells producing inflammatory cytokines [8] have all been implicated as major contributors to IBD progression. Further, it is well known that the mucosa of CD patients is dominated by the Th1 phenotype and is characterized by the production of IFN-γ by lamina propria (LP) T cells and IL-12 by LP macrophages [9,10].

Chemokines are a recently discovered family of small (8–10 kDa) proteins that play an important role as potent chemoattractants for activation and recruitment of leukocytes [11,12]. They are divided into four subfamilies, C, CC, CXC, and CX3C depending on the position of the first two-cysteine residues [13]. Chemokines have the ability to attract inflammatory cells to IBD lesions and in fact are also involved in the activation of these cells. In addition to T cells, neutrophils, and macrophages, the gut of IBD patients is also infiltrated by an abundance of fibroblasts, endothelial cells, and epithelial cells, which in addition to being recruited by chemokines, can themselves produce many chemokines with prominent roles in inflammatory processes [14]. Thus, chemokines and their receptors orchestrate tissue specific and immune cell selective trafficking and retention of leukocytes at the site of

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inflammation, a process that is known to contribute to the development and progression of IBD.

Both experimental studies in mice and evidence from clinical investigations support a role for various chemokines in IBD pathogenesis. For example, polymorphisms in macrophage inhibitory factor (MIF), a chemokine responsible for recruitment of distinct macrophage populations is associated with risk of IBD [15,16]. Further, we have recently shown that CXCR3 ligands (CXCL9, CXCL10, and CXCL11) expressed by activated T cells and NK cells are upregulated at sites of colitis [17]. Similarly, CXCL10 has been shown to be upregulated during UC [18], while CD tissues have been shown to express CXCR3, CXCL10, and CXCL9 [19,20]. Chemokine CCL25, also known as thymus-expressed chemokine (TECK), a key regulator of leukocyte migration in the small intestine, is known to regulate intestinal inflammation [21]. Monocyte chemoattractant protein 1 (MCP-1) that attracts monocytes among other cells plays a critical role in colitis and is increased in IBD patients [22,23]. It has also been shown that IL-16 activates expression and production of proinflammatory cytokines such as IL-1β and tumor necrosis factor alpha (TNF-α) in human monocytes and is significantly increased in IBD patients as compared to healthy controls [24].

The current evidence clearly suggests a link between increased levels of chemokines, cytokines and IBD pathogenesis. However, the available human studies are limited by the patient sample size and the number of chemokines/cytokines measured. Therefore, the purpose of this study was to perform an extensive examination of circulating chemokine and cytokine concentrations in IBD patients. Given the role of T helper cells, macrophage and neutrophils in IBD development and progression, in this study we focused specifically on T helper cell, macrophage, and neutrophil mediated chemokines and cytokines.

2. Materials and methods

2.1. IBD patients

A total of 42 age-matched serum samples were collected at Palmetto Health Hospital at Richland Medical Park by surgical collaborator team of Dr. Raja Fayad from a cohort of 24 patients with chronic CD (18 females and 6 males with a mean age of 41.6 years and an age range between 31 and 76), 18 patients with UC (12 females and 6 males – with a mean age of 39.2 years and age range between 30 and 76) and 10 serum samples from healthy donors (6 females and 4 males with a mean age of 47 years and an age range between 38 and 62) over a period of two years. The average body mass index of IBD patients was 21.4 kg/m^2. The diagnosis of IBD was based on standard clinical, endoscopic and histological criteria. All patients had symptomatic active IBD or strictures that required surgical treatment. The serum samples were collected from the IBD patients before any treatment of antibiotics or steroids. Normal, healthy donors had no active gut or intestinal disease or symptoms at the time of blood collection. Formal consent was obtained from the patients, who were informed about the future use of the serum. The study was approved by the institutional review board for the study of human subjects at University of South Carolina and Palmetto Health systems (PH IRB # 2012-094; PRO-00021486).

2.2. Chemokine and cytokine analysis by multiplex™ ELISA

Levels of macrophage, neutrophil, and T helper cell-derived chemokines and cytokines (IL-16, TNF-α, I-309, CXCL6, IFN-γ, IL-1β, MIF, CCL25, CCL23, CXCL5, CXCL13, CXCL10, CXCL11, MCP1 and CCL21) were determined in the serum using a luminex Elisa assay kit (Bio Rad, Hercules, CA, USA). In brief, IL-16, TNF-α, I-309, CXCL6, IFN-γ, IL1β, MIF, CCL25, CCL23, CXCL5, CXCL13, CXCL10, CXCL11, MCP1 and CCL21 analyte beads contained in an assay buffer were added to pre-wet vacuum wells followed by 50 μl of assay buffer with beads. The buffer was then removed from the wells underwent a wash cycle. Next, 50 μl of standard or serum sample was added to each well and the plate was incubated for 1 h and subjected to continuous shaking (at setting #3) using a Lab-Line™ Instrument Titer Plate Shaker (Melrose, IL). The filter bottom plates were then washed and vortexed at 300xg for 30 s. Subsequently, 25 μl of anti-mouse detection Ab was added to each well and incubated for 30 min at room temperature (RT). Next, 50 μl of streptavidin–phycoerythrin solution was added and the plate was again incubated with continuous shaking for 10 min at RT. Finally, after washing 125 μl of assay buffer was added and BioRad™ readings were measured using a Luminex™ System (Austin, TX) and calculated using BioRad software. The Ab BioRad™ MAP assays are capable of detecting >10 pg/ml for each analyte.

2.3. Power and statistical analysis

Power calculations were performed in order to determine the probability $(1-\beta)$ of detecting a significant difference (δ) between systemic levels of the chemokines and cytokines in IBD patients as compared with normal healthy donors. Based on preliminary investigations, we calculated that at least 40 IBD subjects and 10 healthy donors were needed to show significant differences between the groups, with a power of 95%, and consideration of Type 1 Error 0.001. Data are expressed as the mean ± SEM and compared using a two-tailed paired student's *t*-test or an unpaired Mann Whitney *U*-test. The results were analyzed using Microsoft Excel (Microsoft, Seattle, WA) for Macintosh computers. The results were considered statistically significant if p values were < 0.05.

3. Results

3.1. Chemokines increases in inflammatory bowl disease patients

It has been shown that systemic CC chemokines levels are increased in many autoimmune diseases. Therefore, we sought to determine whether any systemic changes in CC chemokine concentrations are characteristic of disease in IBD patients. As hypothesized, we found that serum levels of macrophage migration factor (MIF), thymus-expressed chemokine (TECK: CCL25), macrophage inflammatory protein-3 (MIP3: CCL23), monocyte chemoattractant protein-1 (MCP-1: CCL2), and macrophage inflammatory protein-3 beta (MIP3β/Exodus-2: CCL21) are increased in IBD patients as compared to healthy donors (Fig. 1A) ($P < 0.05$). Surprisingly, however, we noticed a slight increase in serum levels of I-309 (CCL1) in IBD patients as compared to healthy controls (Fig. 1A) ($P < 0.05$). These data indicate that systemic CC chemokines are significantly increased in IBD patients.

3.2. Increased levels of CXC chemokines in inflammatory bowl disease patients

Among CXC chemokines, the role of CXCR3 in chronic inflammation has been intensively investigated in the past. CXCL10, a CXCR3 ligands level is increased during inflammation as reported in UC patients. However, the relationship between other CXC chemokines and IBD has been less well characterized in the literature. Therefore, in the present study we report that serum levels of IFN-γ-Inducible Protein-10 (IP-10: CXCL10), interferon-inducible T-cell alpha chemoattractant (I-TAC: CXCL11),