Increased expression of CXCL16, a bacterial scavenger receptor, in the colon of children with ulcerative colitis

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Ulcerative colitis

Abstract

Background and aims: CXCL16 is a scavenger receptor which has been connected to phagocytosis of bacterial antigens in experimental colitis. It has also been shown to have a pivotal role in the development of experimental colitis in mice. The increased expression of CXCL16 has been demonstrated in inflamed lesions of patients with Crohn disease. Our aim was to study the expression of CXCL16 in the colon of patients with ulcerative colitis.

Methods: Relative quantitative reverse transcription-polymerase chain reaction was applied to explore the gene expressions of CXCL16, its receptor CXCR6, and interleukin 8, an inflammatory marker, in the colonic biopsies of children with active ulcerative colitis (n = 19), children with ulcerative colitis in remission (n = 9) and children with no inflammatory condition in colon (n = 14).

Results: An increased expression of CXCL16 in the colonic biopsies of children with ulcerative colitis was found both in active disease (p = 0.006) and in remission (p = 0.033), when compared to children without inflammatory condition. The gene expressions of interleukin 8 and CXCL16 correlated with each other (r\textsubscript{s} = 0.67, p = 0.01). The expression of CXCR6 mRNA was comparable between the study groups (p = 0.50).

Abbreviations: CD, Crohn disease; IBD, inflammatory bowel disease; IL, interleukin; iNKT cells, invariant natural killer T cells; UC, ulcerative colitis.

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1. Introduction

Crohn disease (CD) and ulcerative colitis (UC) are the two main forms of inflammatory bowel disease (IBD) affecting increasing amounts of people worldwide. Although the precise cause of IBD is unknown, dysbiosis, intestinal microbial imbalance, has been found both in CD and UC. Moreover, abnormal activation of pattern recognition receptors, such as Toll-like and NOD-like receptors, recognizing different microbial structures has been linked to IBD further emphasizing the potential role of microbes in the pathogenesis of IBD.

CXCL16 is a chemokine of the CXC family that is detected on antigen-presenting cells like macrophages and dendritic cells where it functions as a scavenger receptor mediating adhesion of both Gram-negative and Gram-positive bacteria. CXCL16 plays a key role in the phagocytosis of bacterial antigens and T helper 1 (Th1) immune response in experimental colitis. Its increased expression has been found in the inflamed lesions of patients with CD. In addition, soluble CXCL16 serum levels are increased in patients with CD or UC as compared to those in healthy subjects.

There is no published data concerning expression of CXCL16 in colon of patients with UC. Therefore, we decided to evaluate the gene expressions of CXCL16 and its receptor CXCR6 in the colonic biopsies of children with UC.

2. Materials and methods

2.1. Patients

Patients were recruited at the Department of Pediatrics both in Turku University Hospital and Tampere University Hospital. Endoscopies were done on the clinical basis due to previous IBD or symptoms suggestive of IBD. Endoscopic findings in colonoscopy were classified according to Mayo endoscopic subscore as normal or inactive disease (score 0), mild (score 1), moderate (score 2) and severe disease (score 3). Three groups of patients were included in the study: 19 children with endoscopically active UC (active UC), 9 children with clinically and endoscopically quiescent UC (quiet UC) and 14 children with macroscopically and microscopically non-inflamed colon to whom endoscopy was done due to chronic diarrhea (n = 11) or hematochezia (n = 3). Written informed consent was obtained from all the study patients or their parents. The study was accepted by the ethical committee of the hospital district of Southwest Finland.

2.2. RNA isolation, reverse transcription reactions and gene expression assays

One biopsy for RNA isolation was taken from each patient. The biopsy was taken in involved area if macroscopic inflammation was found during endoscopy. Otherwise, the biopsy was taken in non-involved area. In any case, a sample for histological evaluation was taken in the same area as the biopsy for RNA isolation in order to confirm whether the area was inflamed or not. Biopsy samples were rinsed with RNase free water and then immediately immersed in RNA later RNA stabilization reagent (Qiagen, Hilden, Germany). After the tissue homogenization, isolation of RNA was performed by RNeasy Plus Mini-kit (Qiagen) according to the manufacturer’s instructions. Quality of the isolated RNA was analyzed with Bio-Rad Experion System (Bio RAD Laboratories, Hercules, CA).

Reverse transcription reactions were done by High Capacity cDNA reverse transcription kit (Applied Biosystems/Life Technologies Corporation, Carlsbad, CA) as described in detail earlier. Gene expression assays were performed using comparative Ct (threshold cycle)-method with ABI 7300 Real Time PCR System (Applied Biosystems). The following Taqman Gene Expression Assays (Applied Biosystems) were applied: interleukin (IL) 8, assay ID: Hs00174103_m1; CXCL16, assay ID: Hs00222859_m1; CXCR6, assay ID: Hs01890898_s1 and 18S rRNA, assay ID: Hs99999901_s1 according to the kit’s protocol.

The gene expression of 18S RNA was used as an endogenous control due to its constant expression in all the study samples. Thermal cycler conditions were 1) 50 °C for 2 min, 2) 95 °C for 10 min, 3) 95 °C for 15 s, and 4) 60 °C for 1 min. The last two steps were repeated 40 times. A positive control, cDNA of the Universe Human Reference RNA (Agilent Technologies, Santa Clara, CA) and a negative control were included in every PCR run. Results were analyzed with RQ-Study program (Applied Biosystems) and gene expressions in relation to that in the positive control were calculated as previously described.

2.3. Statistics

Data are presented as medians with minimum and maximum or 25th and 75th percentile due to non-normal distribution. Comparisons between three different groups were made by Kruskal–Wallis analysis of variance and between two groups by Mann–Whitney U test. Measurement of association between two variables was done by Spearman’s rank correlation. A p value less than 0.05 was considered statistically significant. All the statistical analyses were made by IBM SPSS Statistics (Version 20.0.0 for Mac OS X).

3. Results

3.1. Clinical characteristics

Clinical characteristics of the study subjects are presented in Table 1. According to the Mayo endoscopic subscore most of the patients with active UC had mild inflammation. C-reactive protein, as an indicator of systemic inflammation, was increased in active UC as compared to control group of children with non-inflamed colon (p = 0.01). Similar
tendency was found between active UC and quiet UC (p = 0.09). Six patients diagnosed with UC at the time of endoscopy were without medications whereas most of the patients with active UC and previous diagnosis of UC were on two or three medications (Table 1).

### 3.2. Gene expressions in colonic biopsies

The relative gene expression of IL-8 mRNA was increased in active UC as compared to controls (p < 0.0001) and quiet UC (p = 0.016) (Fig. 1). The IL-8 mRNA expression was also greater in quiet UC than in controls (p = 0.003). As shown in the Fig. 2, the expression of CXCL16 mRNA was increased in active UC as compared to controls (p = 0.006) but was comparable to quiet UC (p = 0.36). Again, the CXCL16 mRNA expression was greater in quiet UC than in controls (p = 0.033). As shown in Fig. 3, there was a significant correlation between the gene expressions of IL-8 and CXCL16 (rs = 0.67, p = 0.01). The expression of CXCR6 mRNA was comparable between the study groups (p = 0.50) (Fig. 4).

### 4. Discussion

We found that the expression of CXCL16 mRNA, a chemokine and a scavenger receptor for bacteria, was significantly increased in the colonic biopsies of children with UC both in active and non-active diseases as compared to control children with non-inflamed colon. The gene expression of CXCL16 correlated with that of IL-8, a chemokine abundantly expressed in the intestinal mucosa in IBD. These findings suggest an important role of CXCL16 in the pathogenesis of UC. Indeed, CXCL16 was involved in both phagocytosis of bacterial antigens and production of Th1 cytokines interferon gamma and IL-12 in experimental murine colitis. CXCL16 knock-out mice were less susceptible to colonic inflammation than their

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**Table 1** Clinical characteristics of the study subjects.

<table>
<thead>
<tr>
<th></th>
<th>Active UC (n = 19)</th>
<th>Quiet UC (n = 9)</th>
<th>Control (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13 (7–17)</td>
<td>14 (4–16)</td>
<td>8 (3–13)</td>
</tr>
<tr>
<td>Duration of CU (months)</td>
<td>17 (0–81)</td>
<td>23 (12–102)</td>
<td></td>
</tr>
<tr>
<td>Pancolitis</td>
<td>15 (79%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left-sided colitis</td>
<td>4 (21%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayo endoscopic subscore a</td>
<td>1 (1–2)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>C-reactive protein (mg/l) b</td>
<td>2.7 (.1–10.6)</td>
<td>.3 (0–8.7)</td>
<td>.2 (.1–1.7)</td>
</tr>
<tr>
<td>Medication c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6 (32%)d</td>
<td>0</td>
<td>14 (100%)</td>
</tr>
<tr>
<td>5-ASA</td>
<td>13 (68%)</td>
<td>9 (100%)</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>5 (26%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>9 (47%)</td>
<td>1 (11%)</td>
<td></td>
</tr>
<tr>
<td>Infliximab</td>
<td>0 (11%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented either as median (minimum–maximum) or number (percentage value) as appropriate.

a Endoscopic finding in colonoscopy was classified either normal/inactive (score 0), mild (score 1), moderate (score 2) or severe (score 3) as described earlier (10).

b p = 0.01 active CU vs. control (Mann–Whitney U test); p = 0.09 active CU vs. quiet CU (Mann–Whitney U test).

c Four patients with active UC and one with quiet UC were on two medications, whereas five patients with active UC and one with quiet UC were on three medications.

d All the 6 patients diagnosed with UC at the time of endoscopy were without medications.

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**Figure 1** The relative expression of IL-8 mRNA in colonic biopsies. The column represents median and the error bar represents interquartile range. *Kruskal–Wallis analysis of variance, #Mann–Whitney U test.

**Figure 2** The relative expression of CXCL16 mRNA in colonic biopsies. The column represents median and the error bar represents interquartile range. *Kruskal–Wallis analysis of variance, #Mann–Whitney U test.
wild-type littermates. In addition, administration of CXCL16 monoclonal antibodies improved the condition in two different colitis models further supporting a critical role of CXCL16 in the colonic inflammation.7

Soluble CXCL16 serum levels have been found to be elevated in patients with UC and CD both in active and non-active diseases as compared with that in healthy controls.9 Moreover, serum levels of CXCL16 have been shown to correlate with clinical activities of both UC and CD.7 A previous study demonstrated an increased expression of CXCL16 mRNA in inflamed lesions of adult patients with CD as compared with that in non-inflamed lesions of the same patients.8 However, there were no controls with non-inflamed gut in the study. In agreement with our finding, there was also a significant correlation between the gene expression of IL-8 and that of CXCL16 in these patients.8

IL-8 is a proinflammatory cytokine, which is activated by other proinflammatory cytokines such as tumor necrosis factor-α and interleukin-1β, both found important in the pathogenesis of UC.2 IL-8 is produced by a variety of cells including epithelial cells, monocytes and macrophages.13,14 Increased infiltration of immune cells in lamina propria expressing high levels of CXCL16 has been demonstrated to be the major cause of CXCL16 abundance in patients with colonic CD.8 Therefore, the production of both IL-8 and CXCL16 by the same type of immune cells as an inflammatory response is a possible explanation for the correlation found between the gene expressions of these molecules in this study. IL-8 functions as a chemoattractant and major activator of neutrophils that is thought to result in migration of neutrophils into intestinal tissue from peripheral blood in UC.15 Moreover, lamina propria IL-8 has been found to be proportionately elevated in areas of greater histopathological inflammation in children with UC and CD further supporting its important role in the pathogenesis of IBD.16

Although the exact role of CXCL16 in the pathogenesis of ulcerative colitis is far from clear, a recent experimental murine study offers an attractive clue.17 In the study, colonization of neonatal, but not adult, germ-free mice with a conventional microbiota prevented the animals from oxazolone-induced colitis, a murine model of UC. The preventive effect was associated with a decreased accumulation of invariant (Type I) natural killer T (iNKT) cells in the colonic lamina propria.17 The accumulation, in turn, was directly dependent on CXCL16 expression in colon. Administration of CXCL16 antibodies to mice was able to prevent both the expression of CXCL16 and the accumulation of iNKT cells in colon. Moreover, these mice were protected from colitis-induced pathology and mortality. Of note, the gene expression of the receptor CXCR6, found also on the iNKT cells, was comparable in mice with and without colitis, as was the case here in patients with and without UC. It was further shown that the preventive effect of neonatal colonization on experimental colitis was due to epigenetic suppression of CXCL16 gene expression in colon by a conventional microbiota.17 Similarly, possible effects of microbiota, if any, on CXCL16 gene expression in patients with UC should also be mediated by epigenetic mechanisms since a previous trial found no significant association between the susceptibility or phenotype of UC and the CXCL16 gene polymorphism.18

Interestingly, lamina propria in UC patients was found to be populated by non-invariant (Type II) NKT cells in a recent study.19 These cells responded to lyso-sulfatide glycolipid, a self-antigen, by producing IL-13 and inducing increased epithelial cell cytotoxicity.19 The authors hypothesized that this NKT-cell mediated autoimmune-like response would be one of the first steps in the pathogenesis of UC.19 Prior to that, according to the hypothesis, a defect in the response of intestinal epithelial lining to endogenous microbiome is required.

In conclusion, we demonstrated for the first time that the expression of bacterial scavenger receptor CXCL16 is increased in colon in patients with active and non-active UC. This and previous findings together suggest a pivotal role of CXCL16 in the pathogenesis of UC warranting further studies on the topic.
Conflict of interest

None of the authors have any conflict of interest in relation to the article.

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Statement of authorship

MK designed the study, collected the data, performed the statistical analysis, and drafted the manuscript. SR participated in the collection of colonic biopsies and writing the manuscript. HE participated in the collection of colonic biopsies and writing the manuscript. MA participated in the collection of colonic biopsies and writing the manuscript. TR participated in the collection of colonic biopsies and writing the manuscript. All authors read and approved the final manuscript.

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