

# CXCL12-CXCR4 Axis in Ulcerative Colitis

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## 1. Introduction

Regulated immune responses are essential to maintain intestinal homeostasis and require direct or indirect communication among cells. Communication that occurs among cells in the absence of direct contact is often through the use of cytokines and chemokines. Ulcerative colitis (UC) and Crohn's disease (CD) are chronic intestinal inflammatory bowel diseases (IBD). IBD is characterized by increased influx of immune cells to the mucosa of genetically susceptible hosts. The characteristic increase of inflammatory infiltrate is mainly of T cells recruited to the lamina propria (LP) by a multistep process that involves the integrated interactions and effects of adhesion molecules and chemokines (1). Numerous studies in IBD patients and in animal models of colitis have demonstrated that both inflammatory chemokines and their receptors are up-regulated in settings of active inflammation (2). More importantly, blockade or absence of various chemokine receptors attenuates disease in murine models of IBD. Thus, identifying chemokines and their receptors that are involved in intestinal inflammation provide promising targets for new drug development in the treatment of IBD.

## 2. What is Chemokines?-Role in the pathogenesis of IBD-

Chemokines have been implicated in many fundamental immune processes, including lymphoid organogenesis, immune cell differentiation, development and positioning (3) Chemokines are small 8-12kDa cytokines that can direct the recruitment and migration of circulating leukocytes and play a critical role in the differentiation of secondary lymphoid organs. There are approximately 50 known chemokines and 20 known receptors. Chemokines are classified in 4 separate families based on the pattern of their cysteine residues(C, CC, CXC and CX3C). The CC family of chemokines contains two adjacent cysteine residues. The CXC family has two cysteine residues separated by a non-cystein amino acid, whereas the CX3C family has two cysteine residues separated by three non-cysteine amino acids. The C family has only one cysteine residue. Several reports on the relationship between IBD and chemokine have been reported. Here we will review several

chemokines that have been investigated in the context of IBD and finally described CXCL12-CXCR4 axis including our data.

### **3. CC family of chemokines**

#### **3.1 CCL2 (MCP-1) and CCR2**

CCR2 and its ligands MCP-1,-2,-3 and -4 are involved in the recruitment of monocytes, dendritic cells, and memory T cells. In the intestine, MCP-1 is produced by intestinal epithelial cells. Mice deficient in MCP-1 are protected from hapten-induced colitis, as demonstrated by reduced histological scores of colitis and lower IL-1 $\beta$ , IL-12p40, and IFN- $\gamma$  (4). Furthermore, CCR2-deficient mice with dextran sulfate sodium (DSS)-induced colitis had lower histological scores than wild type mice (5). The chemokine receptor antagonist TAK-779, which blocks CCR2, CCR5, and CXCR3 could reduce colonic inflammation of DSS-induced colitis (6). These data suggested that CCR2 and its ligands seemed to play a crucial role in intestinal inflammation.

#### **3.2 CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES) and CCR5**

Expression of CCL3 is up-regulated in the colon of rats exposed to TNBS and Administration of neutralizing antibodies to CCL3 blocked massive neutrophil influx (7). Expression of RANTES is induced by TNF- $\alpha$  and IFN- $\gamma$  and, together with its receptors CCR1 and CCR5, is up-regulated in the chronic phase of TNBS-induced colitis in rats (8).

Interestingly, RANTES was specifically expressed in non-caseating granulomas of CD patients by in situ hybridization with surrounding CD4<sup>+</sup> T cells expressing the CCR5 and CXCR3 receptors. CCR5 and its ligands are involved in the migration of T cells and monocytes (9). CCL5-deficient mice are less susceptible to DSS-induced colitis and the inflammation that appears in CCR5-deficient mice is characterized by increased CD4<sup>+</sup>T cell and NK1.1<sup>+</sup> cell influx together with an up-regulation of the Th2 cytokines IL-4, IL-5 and IL-10 (4).

#### **3.3 CCL20 and CCR6**

CCL20 mediates chemotaxis of T cells, B cells and DCs (3). In the intestine, CCL20 is produced from IECs. In human IBD, CCL20 protein and RNA expression was increased in intestinal tissues of patients with CD but not those with UC. CCR6 deficient mice leads to decreased susceptibility to DSS (10) and intravital microscopic analyses showed that CCR6 blockade on T and B cells reduced their adherence to mucosal and submucosal microvessels in the course of DSS colitis. Recently, CCR6 has been identified as a key modulator of Th17 cell recruitment to the intestine (11). Taken together, these data may suggest that CCL20 and CCR6 have a chemotactic effect on T and B cells under inflammatory conditions in the colon.

#### **3.4 CCL25 (thymus-expressed chemokine, TECK) and CCR9**

CCL25 is constitutively expressed by thymic epithelial cells and IECs in the small intestine but not in the colon (12). CCL25 binds to the CCR9 expressed on T cells and IgA<sup>+</sup> plasma cells. In the intestine, CCR9 is expressed by both  $\alpha\beta$  and  $\gamma\delta$  CD8 $\alpha\alpha$ +

intraepithelial lymphocytes (IELs) and these cells migrate towards CCL25. CCR9-deficient mice have a remarkable reduction of  $\gamma\delta$  IELs and administration of neutralizing antibody against CCL25 to young mice leads to decreased  $\alpha\beta$  and  $\gamma\delta$  CD8 $\alpha\alpha$ + IELs, suggesting that CCL25-CCR9 pathway might be involved in the early stages of intestinal inflammation.

#### **4. CX3CL1 (fractalkine) and CX3CR1**

CX3CL1/fractalkine is a member of the CX3C chemokine family, which is expressed by epithelial cells and IECs (13). CX3CL1 can be up-regulated by TNF- $\alpha$ , IL-1 $\beta$ , LPS and IFN- $\gamma$ . CX3CL1 can function as an endothelial adhesive determinant to recruit a sub-population of dendritic cells and macrophages that have high CX3CR1 expression. The association between CX3CL1 and pathogenesis of IBD remains controversial. However, Sans, et al. reported that circulating T cells and lamina propria T cells from active CD patients contained a higher proportion of CX3CR1+ cells than CD patients with inactive disease or healthy subjects (14).

### **5. The CXC family of Chemokines**

#### **5.1 CXCL5 (ENA-78) and CXCR2**

Epithelial cell-derived neutrophil-activating peptide(ENA)-78 is a potent neutrophil chemoattractant, which is produced from IECs and LPS, TNF- $\alpha$  and IL-1 $\beta$  stimulate its production. ENA-78 shares sequence homology with CXCL-8 (IL-8). Expression of ENA-78 is induced in the colonic tissues of both patients with UC and CD (15, 16).

#### **5.2 CXCL8 (IL-8)**

IL-8 is also a neutrophil chemoattractant that is produced by macrophages, fibroblasts, epithelial cells, hepatocytes and endothelial cells (17). Up-regulation of IL-8 in the gut of both UC and CD was observed and IL-8 production appears to correlate with histological severity of disease (18). As well as ENA-78, IL-8 can bind CXCR2, although its affinity for the receptor is lower.

#### **6. CXCL12 (SDF-1) and CXCR4**

Among chemokines, CXC chemokine ligand (CXCL)12 (stromal cell-derived factor (SDF)-1/pre-B-cell-growth-stimulating factor (PBSF)) is particularly intriguing because it has been shown definitively to be involved in various developmental processes including hematopoiesis (19,20). CXCL12 was first characterized as a pre-B-cell growth-stimulating factor and the primary physiologic receptor for CXCL12 is CXCR4, which also functions as an entry receptor for strains of HIV-1(21). The studies using mutant mice with targeted gene disruption have revealed that CXCL12 and CXCR4 are essential for B cell development and colonization of bone marrow by hematopoietic stem cells (HSCs) and myeloid lineage cells during ontogeny as well as blood vessel formation in gastrointestinal tract, cardiac ventricular septum formation, and cerebellar development (22, 23,24). Recently, it was reported that CXCL12-CXCR4 chemokine signaling is essential for the development of plasmacytoid dendritic cells (pDC), which could rapidly produces

a huge amount of type I IFN ( $\alpha$ ,  $\beta$ ) following microbial stimulation (25), This axis also play a crucial role in the development of natural killer (NK) cells, which are generated from hematopoietic stem cells and play vital roles in the innate immune response against viral infection (26). Thus, CXCL12-CXCR4 axis is widely involved in the development of immune cell. The concept of CXCL12 as being solely a constitutive chemokine was recently challenged by data from other groups that investigated immune-mediated inflammatory disorders and demonstrated its role in joint, lung, and liver inflammation (27, 28).

Nanki et al. showed that CXCL12 is highly expressed in the synovium of RA patients in contrast to patients with OA, and that anti-CD40 stimulation enhanced CXCL12 production by cultured synoviocytes from RA patients (29). Those authors hypothesized that the CD40 ligand (CD154) expressed on activated memory T cells may stimulate the production of CXCL12 by synoviocytes and increase the migration of T cells.

Thus, CXCR4/SDF-1 $\alpha$  and its ligand CXCL12 is an important chemokine/receptor pair in various diseases, but have received very little attention in IBD.

## 7. How is CXCL12/CXCR4 axis involved in the pathophysiology of IBD?

Mikami, et al. investigated the role of CXCL12/CXCR4 axis in patients with IBD by analysis of CXCR4 expression on peripheral T cells (30). They demonstrated that CXCR4 expression on peripheral T cells in patients with active UC was significantly higher than that in inactive UC and controls. Moreover, a significant correlation between CXCR4 expressions and disease activity in patients with UC was observed. Hosomi, et al focused on the role of immature plasma cells in the pathophysiology of IBD (31). They demonstrated that the proportion of immature plasma cells was correlated positively with clinical activities of UC and CD and expression of CXCR3 and CXCR4 of immature plasma cells in UC patients was significantly higher than in controls. In addition, Dotan, et al. reported that CXCR4 was expressed by intestinal epithelial cells (IECs) and lamina propria cells and CXCR4 positive cells are significantly increased in lamina propria of IBD (32). Moreover, recent report indicated that evaluation of CXCR4 expression on CD4 T cells by FACS analysis could be a biomarker of Leukocytapheresis with a leukocyte removal filter (Cellsoba; Asahi Medical, Tokyo, Japan) (33). These data strongly suggested that CXCR4 positive cells could be involved in the pathophysiology of IBD.

As for expression of CXCL12 expressing cells in patients with IBD, Dotan et al. reported as follows: CXCL12 expression of normal intestinal mucosa was more limited to the surface epithelium, while the expression was enhanced and more diffuse in IBD mucosa (32). This up-regulation was specific to IBD mucosa, and did not occur in non-IBD inflammatory conditions. Moreover, in IBD, CXCL12 was significantly up-regulated in the inflamed compared to the non-inflamed epithelium and stronger expression of CXCL12 in intestinal tissues was observed in patients with UC than in those with CD. Thus, this expression was likely to be more specific in active UC patients than in CD patients,

However, using CXCL12/GFP knock-in approach, Mikami, et al. revealed that CXCL12-expressing cells were mainly observed in the perivascular sites of the normal colonic mucosa, suggesting that the CXCL12-expressing cells were morphologically considered to be pericytes (adjacent to the endothelial cell) but not epithelial cells (30). After DSS

administration, gene expression of CXCL12 was strongly induced in mice with DSS-colitis, which was compatible to human IBD data reported by Dotan. In this regard, more investigation should be required to identify CXCL12 expressing cells in normal and pathogenic conditions of intestinal mucosa. However, the exact reason why CXCL12-CXCR4 signaling was specifically involved in the pathophysiology of UC remains unclear.

## 8. Blockade of CXCL12-CXCR4 signaling as therapeutic target of IBD

As mentioned above, this axis can be strongly involved in the pathophysiology of IBD, particularly, UC. Next question is whether or not blocking of CXCL12-CXCR4 axis can be a new therapy for IBD. Mikami, et al showed that the effect of CXCR4 antagonist (TF14016) on colitis in dextran sodium sulfate (DSS) and interleukin (IL)-10 knockout (KO) models (30).

Firstly, they examined CXCR4 expression on peripheral blood cells and CXCL12 expression of colonic tissue in mice with DSS-induced colitis. As expected, CXCR4 expression of CD4 positive cells was significantly increased after the start of DSS administration, compared with normal mice and gene expression of CXCL12 was also significantly higher in the colonic tissue of mice with DSS-induced colitis than that of normal mice. Next, they evaluated the effect of CXCR4 antagonist (TF14016) on DSS-induced colitis. TF14016 clinically and histologically attenuated intestinal inflammation of DSS-induced colitis. Interestingly, immunohistochemical analysis revealed not only the improvement of colonic inflammation but also reduction of lymphoid aggregations. They also investigated the effect of TF 14016 on cytokine production from mesenteric lymph node cells. Surprisingly, TF 14016 treatment reduced pro-inflammatory cytokine production such as TNF- $\alpha$  and IFN- $\gamma$  but did not alter IL-10 production.

Why did not TF 14016 treatment affect IL-10 production? It should be noted that TF 14016 administration did not alter the percentage of FOXP3+CD25+T cell. This data suggested that ameliorating action of TF 14016 on DSS-induced colitis is mainly due to its inhibitory effect of CD4+CD25- T cells with increased CXCR4 expression. TF 14016 treatment also ameliorated colonic inflammation of IL-10 KO mice.

## 9. Conclusion

CXCL12 and CXCR4 have a constitutive and inflammatory role in the intestinal mucosa. Several human and mouse data strongly suggested that CXCL12-CXCR4 axis play a crucial role in the pathophysiology of IBD, especially UC. Therefore, we hope that therapeutic manipulation of this signaling is considered in IBD therapy.

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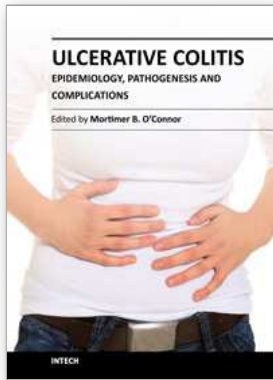
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