Phenotype and Effector Function of CC Chemokine Receptor 9-Expressing Lymphocytes in Small Intestinal Crohn's Disease

Masayuki Saruta, Qi T. Yu, Armine Avanesyan, Phillip R. Fleshner, Stephan R. Targan and Konstantinos A. Papadakis

*J Immunol* 2007; 178:3293-3300; doi: 10.4049/jimmunol.178.5.3293

http://www.jimmunol.org/content/178/5/3293

**References**

This article cites 26 articles, 12 of which you can access for free at: http://www.jimmunol.org/content/178/5/3293.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

Copyright © 2007 by The American Association of Immunologists All rights reserved.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Phenotype and Effector Function of CC Chemokine Receptor 9-Expressing Lymphocytes in Small Intestinal Crohn’s Disease

Masayuki Saruta, Qi T. Yu, Armine Avanesyan, Phillip R. Fleshner, Stephan R. Targan, and Konstantinos A. Papadakis

CCL25/CCR9 chemokine ligand/receptor pair has been reported to play an important role in small bowel (SB) immunity and inflammation. We have previously reported an aberrant SB expression of CCL25 in Crohn’s disease (CD) and an increased frequency of CCR9+ T cells in the peripheral blood of patients with SB inflammatory diseases such as CD and celiac disease. In this study, we have characterized the phenotype and effector function of CCR9+ T cells in mucosal lymphoid tissues in CD. We show that CCR9+ T cells isolated from mesenteric lymph nodes (MLN) draining CD SB express a more activated phenotype compared with MLN draining normal SB. Stimulation of CCR9+ T cells isolated from CD SB lamina propria produced more IFN-γ and IL-17 in response to anti-CD3 or IL-12/IL-18 stimulation compared with those isolated from normal SB. The addition of TL1A to the cytokine combination markedly augmented the secretion of IFN-γ, but not IL-17, by CD lamina propria CCR9+ T cells. CCL25 incubation of CD SB lamina propria lymphocytes and MLN lymphocytes increased their adhesion to VCAM-1/Fc in vitro. Finally, the TCRβ analysis of CCR9+ T cells revealed a diverse TCRβ repertoire among MLN CCR9+ T cells in patients with SB CD. Our data indicate that CCR9+ T cells in SB CD are proinflammatory and support the rationale for the use of CCR9 antagonists for the treatment of human SB CD. The Journal of Immunology, 2007, 178: 3293–3300.

Crohn’s disease (CD) is a chronic, relapsing and remitting inflammatory intestinal disorder that can affect any part of the gastrointestinal tract but often involves the small bowel (SB), colon, or both (1). Although CARD15/NOD2 gene variants are associated with ileal location of CD (2, 3), the pathogenesis of inflammatory bowel disease (IBD). Chemokines and their receptors as well as adhesion molecules that are involved in regulating these processes represent novel therapeutic targets for human IBD (4–6).

Chemokines constitute a large family of small (~8–14 kDa), structurally related heparin binding proteins, which are constitutively expressed in lymphoid and extra lymphoid tissues, and play a critical role in immune homeostasis by regulating lymphocyte trafficking to or within lymphoid organs and in peripheral tissues (7–13). They are classified as C, CC, CXC, and CX3C based on their primary structure and the number of C-terminal cysteine residues. CCL25/CCR9 is the only chemokine receptor pair that has been shown to play a critical role in immune homeostasis by regulating lymphocyte trafficking to or within lymphoid organs and in peripheral tissues (7–13). They are classified as C, CC, CXC, and CX3C based on the positioning of cysteine residues that form two disulfide bonds (14). Chemokines mediate their actions through chemokine receptors on the surface of target cells. Chemokine receptors are currently divided into four families based on the type of chemokine that they bind; they are CXC1 to CXC6, CCR1 to CCR10, XCR1, or CX3CR1 (15).

CCL25 is selectively expressed in the thymus and small intestine but not colon (9, 10). The only known receptor for CCL25, CCR9, is highly expressed on developing thymocytes and small intestinal lamina propria lymphocytes (LPL) and intraepithelial lymphocytes (9, 10, 16). We and others (9, 10, 12) have proposed that the CCL25/CCR9 chemokine ligand receptor pair may play an important role in the regional specialization of intestinal immunity and that the coexpression of CCR9 and αβ of the cell surface may provide a small intestinal “address code” for circulating intestinal memory T cells. We previously reported, that in peripheral blood (PB), CCR9+ T lymphocytes were markedly elevated in patients with SB immune-mediated diseases, including CD, but not in patients with purely colonic CD, and that CCL25 expression is altered in inflamed SB but is not expressed in either normal or inflamed colon. These data suggest the potential involvement of CCR9+ T cells in the pathogenesis of SB CD (11). Furthermore, we have demonstrated that the memory subset of circulating CCR9+ CD4+ T cells in healthy donors has characteristics of mucosal T lymphocytes since they have an activated phenotype, respond to anti-CD2 stimulation, and exhibit a Th1 or T regulatory 1 cytokine profile (12).

In this study, we have characterized the phenotype and effector function of CCR9+ T cells in SB mucosal lymphoid tissues in patients with SB CD and compared it with normal controls. We...
show that CCR9+ T cells isolated from mesenteric lymph node (MLN)-draining CD SB have an activated phenotype and those from CD SB lamina propria (LP) exhibit a predominant Th1 and Th17 cytokine profile. CCR9+ T cells isolated from MLN-draining SB CD express a polyclonal TCRVβ repertoire. In addition, we show that CCL25 triggers the adhesion of CD SB LPL and MLN lymphocytes to VCAM-1/Fc in vitro and therefore could contribute to the CCR9+ T cell trafficking to the SB in inflammatory conditions.

**Materials and Methods**

**Patients**

We enrolled patients with CD, ulcerative colitis (UC), or non-IBD gastrointestinal disorders who were hospitalized at the Cedars-Sinai Medical Center and underwent intestinal resection. The study was approved by the...
Cedars-Sinai Medical Center Institutional Review Board (protocol nos. 3202 and 4254).

Abs and reagents
Anti-CD3 and anti-HLA-DR dye-linked mAbs for immunofluorescence studies were obtained from Caltag Laboratories. Anti-CD25, -CD69, -β2 integrin, -CD40L, -OX40, -CTLA, and -CD45RO dye-linked mAbs were obtained from BD Pharmingen. The PE-conjugated anti-cytokine Abs to IFN-γ, IL-4, IL-5, and IL-10 were from BD Pharmingen. The AlexaFluor488 anti-cytokine Ab to IL-17 was from eBioscience. The anti-CCR9 mAb, 3C3, was from Millennium Pharmaceuticals. Recombinant human VCAM-1/Fc chimera (catalog no. 862-VC) was from R&D Systems. IL-12 and IL-18 were purchased from PeproTech. TL1A was from Human Genome Sciences (17).

Isolation of MLN and LP lymphocytes
Lymphocytes were isolated from MLN draining involved SB from CD patients undergoing surgical resection following mechanical disruption and release of cells in complete RPMI (RPMI 1640 containing 2 mM l-glutamine, 1% nonessential amino acids, 1% sodium pyruvate, 50 μg/ml penicillin/streptomycin, and 10% heat-inactivated FCS). MLN-draining normal SB were isolated from three patients undergoing resection for cancer (2) or polyposis syndrome (1). We also studied the phenotype of MLN draining isolated from seven CD, five UC, or three non-IBD colonic resection specimens.

LPL were isolated from SB CD intestinal specimens or normal SB as described previously (11). Briefly, the intestinal specimen was washed with HBSS and the mucosa was dissected away from the underlying layers. The mucosa layer was incubated in a shaking water bath (100 rpm) in calcium- and magnesium-deficient HBSS, containing 1 mM EDTA, 50 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml amphotericin B, with the solution changed every 30 min until the supernatant was free of epithelial cells. The remaining LP was minced into 1- to 2-mm pieces and was digested for 10 min in RPMI 1640 containing 10% FCS, 0.5 mg/ml collagenase B (Boehringer Mannheim), 1 mg/ml hyaluronidase (Sigma-Aldrich), 0.1 mg/ml DNase I (Sigma-Aldrich), 50 μg/ml gentamicin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml fungizone, in a shaking water bath (100 rpm). The supernatants was collected, filtered through 110-μm nylon mesh (Spectrum Laboratory Products), and centrifuged at 500 × g for 5 min. The cell pellet was resuspended in 15 ml of solution and was centrifuged at 30 × g for 5 min to remove epithelial and other large cells. The supernatant was removed, and lymphocytes were isolated by separation on Ficoll-Hypaque gradients. The cells were then washed three times with HBSS and resuspended in complete RPMI.

FACS analysis
For staining of cell surface Ags, 5 × 10^6 freshly isolated LPL or MLN lymphocytes were washed twice with PBS supplemented with 0.1% BSA and 0.1% azide and resuspended in 100 μl of 10% human AB serum to block nonspecific Fc binding for 15 min. The cells were incubated with the anti-CCR9 mAb 3C3 for 30 min on ice, washed with PBS/BSA/azide, and incubated with a secondary goat anti-mouse IgG2b-TC for 30 min on ice. The cells were washed again with PBS/BSA/azide and incubated with mouse IgG for 15 min. FITC- and PE-conjugated mAb for surface Ag were used for 30 min. After washing twice, cells were resuspended in 400 μl of 1% paraformaldehyde in PBS and analyzed by FACS. A total of 3 × 10^4 events was routinely collected and analyzed using CellQuest software (BD Immunocytometry Systems).

Analysis of intracellular cytokines
For cytokine detection at the single-cell level, LPL from inflamed or normal SB were stimulated with 50 ng/ml PMA (Sigma-Aldrich) and 1 μg/ml ionomycin for 5 h. Brefeldin A (10 μg/ml) or monensin (2 μM) was added to the culture after 2 h of stimulation to block cytokine secretion. Cells were surface stained with CD3-FITC and CCR9-TC, fixed, and permeabilized using Cytofix/Cytoperm solution (Caltag Laboratories). The cells were then stained with PE- or AlexaFluor488-conjugated Abs to IFN-γ, IL-10, IL-4, IL-5, or IL-17 or their isotype controls. After an additional wash step, cells were analyzed by flow cytometry.

Analysis of IFN-γ and IL-17 production by ELISA
IFN-γ was quantitated in culture supernatants by amplified sandwich ELISA as reported previously (12). Briefly, 96-well microtiter plates were coated overnight with anti-IFN-γ mAb (BD Pharmingen). After blocking in PBS/BSA, diluted standards (recombinant human IFN-γ; R&D Systems), and samples were added for 24–72 h and detected by a second anti-IFN-γ biotinylated mAb (BD Pharmingen) for 2 h. After washing, this biotinylated mAb was detected by streptavidin-alkaline phosphatase (Jackson ImmunoResearch Laboratories) for 30 min, followed by four phosphate-free washes. Substrate (0.2 mM NADPH; Sigma-Aldrich) was added for 30 min, then the NADH signal was amplified using 2-propanol (3%) with iodonitrotetrazolium violet (1 mM), alcohol dehydrogenase (75 mg/ml), and diaphorase (50 mg/ml, the last three from Sigma-Aldrich), and plates were read at 490 nm on an Emax plate reader (Molecular Devices). Sample concentration was calculated from a standard curve generated by our own software (R. Deen).

IL-17 was quantitated in culture supernatants by amplified sandwich ELISA. Ninety-six-well microtiter plates were coated overnight with anti-IL-17 mAb (eBioscience). After blocking in assay diluent (eBioscience), diluted standards (recombinant human IL-17; eBioscience) and samples were added for 24–72 h and detected by a second anti-IL-17 biotinylated mAb (eBioscience) for 2 h. After washing, this biotinylated mAb was detected by avidin-HRP (eBioscience) for 30 min. Substrate (tetramethylbenzidine solution; eBioscience) was added for 15 min followed by 1 M peroxidase (Jackson ImmunoResearch Laboratories) for 30 min, followed by four phosphate-free washes. Substrate (0.2 mM NADPH; Sigma-Aldrich) was added for 30 min, then the NADH signal was amplified using 2-propanol (3%) with iodonitrotetrazolium violet (1 mM), alcohol dehydrogenase (75 mg/ml), and diaphorase (50 mg/ml, the last three from Sigma-Aldrich), and plates were read at 490 nm on an Emax plate reader (Molecular Devices). Sample concentration was calculated from a standard curve generated by our own software (R. Deen).

Table I. IFN-γ and IL-10 production by CCR9+ T cells from normal and CD SB

<table>
<thead>
<tr>
<th></th>
<th>% CCR9+IFN-γ a,b</th>
<th>MFI b</th>
<th>% CCR9+IL-10 a,b</th>
<th>MFI b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal SB (n = 5)</td>
<td>47.8 ± 4</td>
<td>46.5 ± 12</td>
<td>7.2 ± 1.8</td>
<td>40.5 ± 18</td>
</tr>
<tr>
<td>CD SB (n = 5)</td>
<td>56 ± 11</td>
<td>57.8 ± 24</td>
<td>8.2 ± 4</td>
<td>40.1 ± 29</td>
</tr>
<tr>
<td>p Value</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.49</td>
</tr>
</tbody>
</table>

a LPL were isolated from normal and CD SB as described in Materials and Methods and activated for 5 h with PMA plus ionomycin. After 2 h of stimulation, brefeldin A (for IFN-γ staining) or monensin (for IL-10 staining) was added. The cells were stained for CCR9-TC and CD3-FTC and intracellular IFN-γ-PE or IL-10-PE and analyzed by flow cytometry.

b The data represent the mean percentage ± SD of cytokine-positive cells and the MFI for IFN-γ and IL-10 in CCR9+ T cells from normal and CD SB.
CD3+CCR9+ cells, and individual TCR families were evaluated based on T cells showing exclusive staining for either FITC or PE or double staining for both FITC and PE. Thus, 24 individual TCR Vβ families can be analyzed in eight individual tubes.

**Statistical analysis**

Differences between the percentage of phenotypic markers or cytokine producing cells between CCR9+ and CCR9- T cells were compared with a t test. Values of p < 0.05 were considered statistically significant.

**Results**

**CCR9+ T cells from MLN draining inflamed SB have an activated phenotype**

To determine the potential involvement of CCR9+ T lymphocytes in SB CD, we analyzed the phenotype of CCR9+ T cells isolated from normal and inflamed SB LPL. LPL were isolated from normal or CD SB and stained for CD3, CCR9, and for a panel of activation markers and costimulatory molecules and analyzed by FACS. As shown in Fig. 1, we observed no major differences in the phenotype of CCR9+ T cells isolated from CD vs normal SB. In both normal and CD SB, most T cells express CCR9 (Fig. 1, B and C), but a small percentage of them coexpress CD25, OX40, surface CTLA-4, or CD40L. HLA-DR was expressed by a subset of CCR9+ T cells from both normal and CD SB, whereas CD69 was highly expressed by most CCR9+ T cells from both normal and CD SB (Fig. 1). Most CCR9+ T cells in both normal and CD SB also expressed CD45RO and β2 integrin (Fig. 1). Therefore, CCR9+ T cells from both CD and normal SB show equal levels of activation.

In contrast, when we analyzed the phenotype of CCR9+ T cells from MLN-draining inflamed CD SB, we found a higher percentage of CCR9+ T cells with an activated phenotype compared with MLN-draining normal SB (Fig. 2). The clearest differences among CCR9+ T cells from inflamed compared with normal SB MLN were seen in the expression of HLA-DR, OX-40, and CD40L. Although the frequency of CD45RO expression by CCR9+ T cells was lower in MLN compared with SB LP (Figs. 1 and 2), there were no differences in marker expression in CCR9+ T cells between MLN draining CD and normal SB (Fig. 2). Our data indicate that T cell activation in MLN-draining SB leads to acquisition of CCR9 and β2 integrin expression, which would target them for homing to the SB mucosa. CCR9+ T cells isolated from MLN-draining SB appear to have a more activated phenotype in SB CD compared with normal SB. In contrast, we found no clear differences in the phenotypic characteristics of CCR9+ T cells isolated from MLN draining UC, CD, or normal colon, although there was a trend for increased frequency of OX40 and CD40L in UC CCR9+ T cells (Fig. 3).

**CCR9+ T cells isolated from inflamed SB have a Th1 and Th17 cytokine profile**

We have previously reported that CCR9+ T cells isolated from PB and normal SB have a prominent Th1 cytokine profile as they...
secreted large amounts of IFN-γ, whereas a small subset of CCR9+IFN-γ-pb T cells also produces IL-10 (10, 12). Therefore, we examined whether CCR9+ T cells isolated from CD SB LP have a predominant Th1 cytokine profile and whether they also produce IL-17 because the IL-23/IL-17 pathway has been associated with the pathogenesis of several autoimmune diseases, including experimental colitis. CCR9+ T cells from either normal or CD SB LPL contained a higher percentage of IFN-γ-producing cells compared with CCR9- T cells (data not depicted). The percentage of CCR9+ IFN-γ-producing cells was slightly higher in SB CD LPL compared with normal SB LPL (56 ± 11 vs 47.8 ± 4%), but the difference was not statistically significant (Table I and Fig 4A). The percentage of CCR9+IL-10-, CCR9+IL-4-, and CCR9-IL-5+ cells among these two groups was also similar and significantly lower compared with the percentage of IFN-γ-producing cells (Tables I and II and Fig 4B).

The percentage of CCR9+ IL-17-producing cells were similar in SB CD LPL compared with normal SB LPL (10.6 ± 5.0 vs 10.1 ± 1.7, p = NS) (Table II and Fig 4A). The CCR9+IL-17+ T cells were largely distinct from the IFN-γ-producing cells, whereas a small percentage of CCR9+ T cells were IFN-γ+IL-17+ double-positive cells (2.6 ± 1.4% in CD LPL vs 3.5 ± 1.8% in normal SB LPL; p = 0.37) (Fig 4A). Our data indicate that CCR9+ T cells isolated from normal and CD SB have a dominant Th1 cytokine profile, but there were no overall differences in the percentage of IFN-γ-producing cells or the mean fluorescence intensity (MFI) of intracellular IFN-γ staining between CD and normal SB CCR9+ T cells when they are maximally activated with PMA plus ionomycin. In addition, ~10% of CCR9+ T cells produce IL-17 and are most distinct from IFN-γ-producing cells in both normal and CD SB.

We next analyzed the accumulation of IFN-γ protein in culture supernatants from sorted CCR9+ T cells isolated from CD vs normal SB LPL. As shown in Fig 5A, CCR9+ T cells isolated from CD LPL secrete significantly more IFN-γ compared with CCR9- T cells isolated from normal LPL when activated with anti-CD3 plus anti-CD28 Abs or with the cytokines IL-12 and IL-18. Interestingly, the addition of TL1A, which was recently shown to synergize with IL-12 and IL-18 to enhance IFN-γ production by CCR9+ PB and LP T cells (13), significantly enhanced the secretion of IFN-γ by CCR9+ T cells isolated from inflamed CD SB compared with CCR9- T cells isolated from normal SB (Fig 5A). The maximum amount of IFN-γ released from CCR9+ T cells (SB CD, 141 ng/ml vs normal SB, 29 ng/ml, n = 4, p = 0.01) was observed when CCR9+ T cells were stimulated with IL-12 plus IL-18 plus TL1A (Fig 5A).

Furthermore, we analyzed the accumulation of IL-17 protein in culture supernatants from sorted CCR9+ T cells isolated from CD

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** A, IFN-γ production by CCR9+ T cells from normal and CD SB lymphocytes. SB LPL were isolated, and CD3+CCR9+ T cells were sorted by FACS and stimulated with plate-bound anti-CD3 and soluble anti-CD28, soluble anti-CD2 and anti-CD28, or IL-12 (1 ng/ml) and IL-18 (50 ng/ml) with or without TL1A (100 ng/ml) or left unstimulated. Forty-eight hours later, supernatants were collected and analyzed for IFN-γ content by ELISA. The mean ± SD of IFN-γ concentration from four donors in inflamed and normal SB is shown. B, IL-17 production by CCR9+ T cells from normal and CD SB lymphocytes. Supernatants collected from the experiments described in A were also analyzed for IL-17 protein content by ELISA.

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** CCL25 triggers the adhesion of SB CD LPL and MLN lymphocytes to immobilized VCAM-1/Fc in vitro. Isolated SB CD LPL and MLN lymphocytes were added to 4-mm VCAM-1/Fc-coated well glass slides as described in Materials and Methods. After washing, adherent cells were counted in five randomly selected HPF with microscopy. The data shown represent the mean number of adherent cell per HPF ± SD. One representative experiment from three different donors is shown.
vs normal SB LPL. As shown in Fig. 5B, CCR9+ T cells isolated from CD LPL secrete significantly more IL-17 compared with CCR9+ T cells isolated from normal LPL, especially when stimulated with anti-CD2 plus anti-CD28 Abs or anti-CD3 plus anti-CD28 Abs. Unlike with IFN-γ production, the addition of TL1A had no effect on IL-17 production by CCR9+ T cells isolated from inflamed CD SB or normal SB (Fig. 5B). The average amount of IL-17 released from CCR9+ T cells in SB CD was 15 ± 7.6 vs 1.8 ± 0.8 ng/ml in normal SB (n = 4, p = 0.01) when CCR9+ T cells were stimulated with anti-CD2 plus anti-CD28 Abs (Fig. 5B).

**TECK/CCL25 triggers the adhesion of SB CD MLN lymphocytes and LPL to VCAM-1**

We have previously reported altered CCL25 expression in inflamed CD SB compared with normal SB by immunohistochemistry and also an increased frequency of CCR9+ T cells in the circulation of patients with SB CD. Since chemokines play a critical role not only in lymphocyte migration but also in integrin activation (18–20), we hypothesized that the aberrant CCL25 expression may contribute to SB homing of effector CCR9+ T cells. Unlike with IFN-γ production, the addition of TL1A had no effect on IL-17 production by CCR9+ T cells isolated from inflamed CD SB or normal SB (Fig. 5B). The average amount of IL-17 released from CCR9+ T cells in SB CD was 15 ± 7.6 vs 1.8 ± 0.8 ng/ml in normal SB (n = 4, p = 0.01) when CCR9+ T cells were stimulated with anti-CD2 plus anti-CD28 Abs (Fig. 5B).

**CCR9+ T cells from the MLN draining CD SB display a diverse TCRVβ repertoire**

We next hypothesized that CCR9+ T cells may expand secondarily to specific antigenic stimulation in draining MLN and therefore may be associated with a restricted expression of TCRVβ families. We used the IOTest β Mark kit (Beckman Coulter), a flow cytometry-based assay for quantitative analysis of the TCRVβ repertoire of human T lymphocytes, to determine the frequency of 24 Vβ families covering ~70% of the TCRVβ repertoire of human T lymphocytes. We found that SB CD MLN lymphocytes express a diverse set of TCRVβ among both CCR9+ and CCR9− T cells (Fig. 7). Interestingly, subsets of TCRVβ, such as Vβ1, Vβ11, and Vβ13.6, were overrepresented among CCR9+ compared with CCR9− T cells (Fig. 7). These changes in the frequency of Vβ1, Vβ11, and Vβ13.6 were significant at the p < 0.05 level.

**Discussion**

In this study, we have examined the phenotype and cytokine profile of mucosal CCR9+ T cells in patients with SB CD. We found that a higher percentage of CCR9+ T cells isolated from MLN draining CD SB, but not the LP, express the activation markers OX40, CD40L, and HLA-DR compared with MLN-draining normal SB. Although the percentage of CCR9+ T cells was lower in MLN-draining CD SB than normal SB in our previous study (11), there appears to be a variability in the detected frequency of CCR9 expression among SB MLN T cells in CD possibly related to the stage of the inflammatory process or the concomitant use of medications. Nevertheless, CCR9+ T cells isolated from MLN draining inflamed CD SB have a more activated phenotype than those isolated from normal SB MLN, whereas CCR9+ T cells from both CD and normal SB show equal levels of activation. The fact that we found no clear differences in the phenotypic characteristics of CCR9+ T cells isolated from MLN-draining UC, CD, or normal colon indicates that these differences are specific to SB CD.

We have previously shown that CCR9+ T cells isolated from normal SB exhibit a prominent Th1 cytokine profile since they mainly produce IFN-γ (10). In this study, we demonstrate that LP CCR9+ T cells isolated from CD as well as normal SB produced mainly IFN-γ but little IL-10 as assessed by intracellular cytokine staining following in vitro stimulation with PMA plus ionomycin. However, when LP CCR9+ T cells isolated from inflamed CD SB were stimulated with anti-CD3 Abs or IL-12 and IL-18, they accumulated significantly more IFN-γ in culture supernatants compared with normal SB. Similar findings we observed in regards to IL-17 production by CCR9+ T cells. Approximately 10% of LP CCR9+ T cells in both CD and normal SB produced IL-17, and most were distinct from IFN-γ-producing cells. However, when CCR9+ T cells isolated from inflamed CD SB were stimulated in vitro with anti-CD3 or anti-CD2 Abs, they accumulated significantly more IL-17 in culture supernatants compared with normal SB (Fig. 5B).

We reported previously that TL1A, a newly discovered cytokine, synergizes with IL-12 and IL-18 to enhance IFN-γ in human T cells, and particularly in PB and SB CCR9+ T cells (13, 17).
this study, we also demonstrate that addition of TL1A to IL-12/IL-18 cytokine combination markedly enhanced secretion of IFN-γ by CCR9⁺ T cells from inflamed CD SB, indicating an important role of TL1A/DR3 interactions in enhancing Th1 immune responses in CD from cytokine stimulated CCR9⁺ T cells. Recently, a similar role of TL1A has been proposed in murine models of SB CD (21). In marked contrast to its effect on IFN-γ production, TL1A had no effect on IL-17 production by cytokine-stimulated CCR9⁺ LP T cells.

Lymphocyte homing to peripheral tissues is controlled by the combinatorial interactions between cell integrins and their respective ligands expressed on endothelial cells (7). Selective homing to mucosal lymphoid tissues is mediated through interactions between αβ integrin and MAdCAM-1 (22). The combined expression of αβ/CCR9 by intestinal homing T lymphocytes may further define SB vs colonic mucosal T cell trafficking through CCL25 (selectively expressed in the SB) triggering of integrin activation from CCR9 engagement (9, 10). Therefore, expression of CCR9 by mucosal homing T cells may be used to distinguish SB from colonic immune responses (12). CCL25 is selectively expressed in the thymus and SB endothelial cells, intestinal crypts, and a subset of LP cells (9, 10). In contrast, there is no expression of CCL25 in normal or inflamed colon (11). The expression of CCL25 by SB endothelial cells could provide the chemokine signal for the firm arrest of CCR9⁺ expressing T cells rolling on SB vascular beds and subsequent extravasation into the LP explaining the preferential recruitment of CCR9⁺ T cells to the SB. A previous study has also reported the aberrant expression of CCL25 in the liver in patients with primary sclerosing cholangitis. CCL25 could trigger adhesion of liver-infiltrating lymphocytes that expressed CCR9 to immobilized MAdCAM-1 in vitro, establishing a link between IBD and primary sclerosing cholangitis through CCR9⁺ T cell-mediated inflammation (23). In this study, we demonstrate that CCL25 triggers the adhesion of CD SB LPL and MLN lymphocytes to VCAM-1/Fc in vitro. CCL25-stimulated LPL or MLN lymphocyte-triggered adhesion to VCAM-1/Fc was as robust as that observed with Mn²⁺, which can directly activate the αβ integrin. Murine studies have implicated an important role for MAdCAM-1, L-selectin, and VCAM-1 in the pathogenesis of experimental ileitis (24, 25), indicating that additional pathways are operative in lymphocyte recruitment in the intestine in inflammatory vs homeostatic conditions. Blocking several integrins/ligands in some experimental models has been shown to be required for effective treatment of experimental IBD (24, 25). This could explain the therapeutic efficacy of natalizumab (anti-α₄ integrin mAb) in CD by inhibiting the shared α₄ integrin moiety of both αβ integrin and αβ integrin in effector cells (6). Therefore, the ability of CCL25 to trigger not only αβ/MAdCAM-1 adhesion (23) but also αβ/VCAM-1 could contribute directly to the adhesion, firm arrest, and subsequent transmigration through the vascular endothelium of effector CCR9⁺ T cells into the SB in CD. Therefore, targeting the CCL25/CCR9 chemokine ligand/receptor pair, which can activate several of these integrins, could prove highly effective for the treatment of human SB CD.

In summary, we have characterized the phenotype and cytokine profile of CCR9⁺ T cells in mucosal lymphoid tissues in patients with SB CD and showed that CCR9⁺ T cells have an activated phenotype in MLN and exhibit a Th1 and Th17 cytokine profile in SB LPL. Additionally, CCL25 can trigger adhesion of CD SB LPL and MLN lymphocytes to VCAM-1/Fc in vitro and therefore could contribute to effector T cell trafficking to the SB in CD. A recent study has indicated the therapeutic potential of a novel small molecule CCR9 antagonist CCX282 (Trafficet-EN; Chemocentryx) in the treatment of experimental ileitis (26).

The presence of activated CCR9⁺ T cells with enhanced Th1 and ThIL-17-producing capacity in human SB CD supports the rationale for the use of selective CCR9 antagonists for the treatment of SB CD.

Acknowledgment

We thank Martin R. Hodge (Millennium Pharmaceuticals) for providing us with the 3C3 anti-CCR9 mAb.

Disclosures

The authors have no financial conflict of interest.

References


