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

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

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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 TCRVβ analysis of CCR9+ T cells revealed a diverse TCRVβ 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.


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Received for publication June 27, 2006. Accepted for publication December 27, 2006.

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This work was supported by grants from the Broad Medical Research Program in Inflammatory Bowel Diseases by the Eli and Edythe L. Broad Foundation (to K.A.P.).

M.S. and Q.T.Y. contributed equally to this work and should be considered as first authors.

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4 Abbreviations used in this paper: CD, Crohn’s disease; HPF, high power field; IBD, inflammatory bowel disease; LP, lamina propria; LPL, lamina propria lymphocyte; MFI, mean fluorescence intensity; MLN, mesenteric lymph node; PB, peripheral blood; SB, small bowel; UC, ulcerative colitis.

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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 ThIL-17 cytokine profile. CCR9+ T cells isolated from MLN-draining SB CD express a polyclonal TCRV 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...
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 detection by avidin-HRP (eBioscience) for 30 min. Substrate (tetramethylbenzidine solution; eBioscience) was added for 15 min following 5 h of incubation 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 (BD Biosciences). A total of 3 × 10^6 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 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 (recombiant 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 NADPH signal was amplified using 2-propanol (3%) with iodonitrotetrazoliun 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. Deem).

IL-17 was quantitated in culture supernatants by 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 (BD Pharmingen) 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 H2O2.

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>% CCR9+ IFN-γ</th>
<th>MFI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% CCR9+ IL-10</th>
<th>MFI&lt;sup&gt;b&lt;/sup&gt;</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 ± 4.3</td>
<td>40.1 ± 29</td>
</tr>
</tbody>
</table>

<sup>a</sup>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-FTTC and intracellular IFN-γ-PE or IL-10-PE and analyzed by flow cytometry.

<sup>b</sup>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 LP. 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 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

Table II.  IL-17, IL-4, and IL-5 production by CCR9⁺ T cells from normal and CD SB

<table>
<thead>
<tr>
<th></th>
<th>% CCR9⁺IL-17⁺</th>
<th>% CCR9⁺IL-4⁺</th>
<th>% CCR9⁺IL-5⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal SB (n = 4)</td>
<td>10.1 ± 1.7</td>
<td>1.9 ± 0.93⁺</td>
<td>0.56 ± 0.15⁺</td>
</tr>
<tr>
<td>CD SB (n = 9)</td>
<td>10.6 ± 5.0</td>
<td>1.7 ± 1.0₁</td>
<td>0.79 ± 0.46⁺</td>
</tr>
<tr>
<td>p Value</td>
<td>0.85</td>
<td>0.80</td>
<td>0.17</td>
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</table>

* 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 and monensin were added. The cells were stained for CCR9-TC and CD3-FITC and intracellular IL-17-AlexaFluor488, or IL-4-PE, or IL-5-PE and analyzed by flow cytometry.

† The data represent the mean percentage ± SD of cytokine-positive cells for IL-17, IL-4, and IL-5 among CCR9⁺ T cells from normal and CD SB. *, three donors; †, seven donors.

FIGURE 4.  Expression of IFN-γ and IL-17 by stimulated CCR9⁺ T cells from normal and CD SB. LPL were isolated from normal and CD SB, SB LPL were isolated as described in Materials and Methods, stimulated with PMA plus ionomycin, and stained for CCR9, CD3, and intracellular IFN-γ and IL-17 (A). Representative dot plot of intracellular IFN-γ and IL-17 staining from a normal and a CD specimen on gated CD3⁺CCR9⁺ T cells is shown. The mean percentage of IFN-γ and IL-17 producing cells among CCR9⁺ T cells in normal and CD SB is shown in Tables I and II. Intracellular cytokine staining for IL-10, IL-4, and IL-5 in normal and SB CD LPL.

H₂PO₄ stop solution. Plates were read at 450 nm on an Emax plate reader (Molecular Devices).

Static adhesion assay

For adhesion assay, 4-mm wells on 18-well glass slides (Cel-Line/Erie Scientific) were coated at 4°C with 10 μl of VCAM-1/Fc (20 ng in 10 μl of PBS). After overnight incubation, VCAM-1/Fc solution was aspirated from the wells, and CCL25 (50 ng in 15 μl of PBS) was added to the wells for 1 h at 37°C. The wells were blocked with 15 μl of FCS for 1 h at 37°C after extensive washing. LPL or MLN lymphocytes were suspended in adhesion buffer (150 mM NaCl, 10 mM HEPES (pH 7.4), 1 mM CaCl₂, and 1 mM MgCl₂) and added to the wells (4.0–4.5 × 10⁴ cells in 15 μl/well). Cells were allowed to adhere for 30 min at 37°C. After washing the slides, adherent cells were counted using microscopy. The number of adherent cells from five high power fields (HPF) per condition was counted and the mean was calculated. Adhesion of cells stimulated directly with adherent cells from five high power fields (HPF) per condition was counted.

Determination of Vβ families by flow cytometry

The IOTest β Mark (TCR Vβ repertoire kit) was obtained from Beckman Coulter. Detection of individual Vβ families was conducted in freshly harvested MLN lymphocytes from patients with SB CD. A total of 5 × 10⁷ cells was stained simultaneously with mAbs directed against CD3-PerCP, CCR9-allophycocyanin, and a set of three Abs directed against TCRVβ families. mAbs directed against TCR families were labeled with either FITC or PE, and the third anti-TCR-directed mAb was labeled with PE and FITC. Flow cytometry was performed using the MoFlo (DakoCytomation). Cells were gated on the CD3⁺CCR9⁺ or
secreted 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 were slightly higher in SB CD LPL compared with normal SB LPL (56 ± 11 vs 47.8 ± 4%), but the difference was not statistically significant (Tables 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 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 most are 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 SB. As shown in Fig. 5B, CCR9+ T cells isolated from CD LPL secrete significantly more IL-17 compared with CCR9+ T cells 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 IL-17 by CCR9+ T cells isolated from inflamed CD SB compared with CCR9+ T cells isolated from normal SB (Fig. 5A).
CD28 Abs. Unlike with IFN-γ, stimulation with anti-CD2 plus anti-CD28 Abs or anti-CD3 plus anti-CD28 Abs did not have an effect on IL-17 production by CCR9+ T cells isolated from patients with active SB CD (n = 5), stained with CCR9allophycocyanin, CD3 PerCP, and a combination of PE, FITC, or PE plus FITC-conjugated Ab against 24 TCRVβ families, and analyzed by flow cytometry. * indicates significant differences (p < 0.05) between CCR9+ and CCR9− T cells.

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+CD3+ 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). In
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 αvβ3 and MadCAM-1 (22). The combined expression of αvβ3/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 LP and MLN lymphocytes to VCAM-1/Fc in vitro. CCL25-stimulated LP or MLN lymphocyte-triggered adhesion to VCAM-1/Fc was as robust as that observed with Mn2+4, which can directly activate the αvβ3 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 α4β1 and α4β7 in effector cells (6). Therefore, the ability of CCL25 to trigger not only α4β1/MadCAM-1 adhesion (23) but also α4β1/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 LP. Additionally, CCL25 can trigger adhesion of CD SB LP 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 Th17-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


