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CCR7 Expression and Memory T Cell Diversity in Humans

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CCR7, along with L-selectin and LFA-1, mediates homing of T cells to secondary lymphoid organs via high endothelial venules (HEV). CCR7 has also been implicated in microenvironmental positioning of lymphocytes within secondary lymphoid organs and in return of lymphocytes and dendritic cells to the lymph after passage through nonlymphoid tissues. We have generated mAbs to human CCR7, whose specificities correlate with functional migration of lymphocyte subsets to known CCR7 ligands. We find that CCR7 is expressed on the vast majority of peripheral blood T cells, including most cells that express adhesion molecules (cutaneous lymphocyte Ag α, β, integrin) required for homing to nonlymphoid tissues. A subset of CD27(neg) memory CD4 T cells from human peripheral blood is greatly enriched in the CCR7(neg) population, as well as L-selectin(neg) cells, suggesting that these cells are incapable of homing to secondary lymphoid organs. Accordingly, CD27(neg) T cells are rare within tonsil, a representative secondary lymphoid organ. All resting T cells within secondary lymphoid organs express high levels of CCR7, but many activated cells lack CCR7. CCR7 loss in activated CD4 T cell subsets accompanies CXC chemokine receptor (CXCR)5 gain, suggesting that the reciprocal expression of these two receptors may contribute to differential positioning of resting vs activated cells within the organ. Lymphocytes isolated from nonlymphoid tissues (such as skin, lung, or intestine) contain many CD27(neg) cells lacking CCR7. The ratio of CD27(neg)/CCR7(neg) cells to CD27(pos)/CCR7(pos) cells varies from tissue to tissue, and may correlate with the number of cells actively engaged in Ag recognition within a given tissue.


The mammalian cognate immune system uses a self/nonself recognition strategy that randomly generates extremely large numbers of receptors, with the assumption that novel Ags will be recognized by at least a few members of the repertoire. As only perhaps 1 in 10^7 T cells may recognize a potentially threatening immunological target, it is imperative that as many lymphocytes as possible be exposed to any foreign Ag. Thus, routine immunosurveillance involves constant recirculation of lymphocytes through tissues. Naive T cells follow a relatively simple path of recirculation from the blood into secondary lymphoid organs, then back into the blood via afferent lymph reviewed in Ref. 1). This cycle continues until the cell at some point (during its time within a secondary lymphoid organ) recognizes an Ag for which its TCR is specific. Once a naive T cell recognizes its cognate ligand, it will differentiate into a memory T cell and may begin to express homing receptors that both enable the cell to home to nonlymphoid tissues and prevent it from homing to other types of tissues.

Along with lymphocyte adhesion molecules, chemokines and their receptors appear to play vital roles in the trafficking cycle of lymphocytes during inflammation and routine immunosurveillance (Refs. 2 and 3; reviewed in Ref. 4). CCR7 is a very important player in the mechanism by which T lymphocytes enter secondary lymphoid organs through high endothelial venule(s) (HEV). T cells unable to receive a signal through CCR7 do not adhere to HEV after the initial tethering and rolling process begins; hence, they are unable to enter lymph nodes or Peyer’s patches (Refs. 5 and 6; reviewed in Ref. 4). Mice lacking the CCR7 gene form small, disorganized lymphoid tissues containing very few T or B cells (7).

Ligands for CCR7 are expressed by the HEV of secondary lymphoid organs (5, 6), by parenchymal cells within T zones of lymph nodes (8), and by endothelial cells at the openings of lymphatic vessels within peripheral tissues (8). In addition, CCR7 expression (or lack thereof) defines a subset of peripheral blood CD4 cells enriched in cells of unique “effector” phenotype (9).

Thus, due to the apparent biological significance of this molecule, we found it of great importance to fully characterize the expression of CCR7 on T lymphocytes during all identifiable phases of T lymphocyte trafficking. We have recently generated a series of anti-CCR7 mAbs, two of which recognize epitopes that correlate directly with functional responsiveness to CCR7 ligands. We report our findings with these new Abs below.

Abbreviations used in this paper: HEV, high endothelial venule(s); CXCR, CXC chemokine receptor; SDF, stromal cell-derived factor; MIP, macrophage-inflammatory protein; SL C, secondary lymphoid-tissue chemokine; BCA-1, Burkitt’s lymphoma receptor-1 ligand chemokine; CLA, cutaneous lymphocyte Ag; RA, rheumatoid arthritis.
Materials and Methods

**mAbs to human CCR7**

L1.2 cells were transfected with human CCR7 and used to immunize mice. mAbs to CCR7 were generated as described (10) (the mAbs were produced and purified by the staff of Millennium Pharmaceuticals, Cambridge, MA).

mAbs that recognized CCR7 transfectants but not wild-type L1.2 cells were tested against the following panel of L1.2 cells transfected with various other known (or suspected) chemokine receptors: CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CCR9, CXC chemokine receptor (CXCR) 1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR5, CXCR1, CR1, Bonzo, BOB, AP1, RDC, AF014958, and LyGPR. No appreciable cross-reactivity was observed for any of these receptor transfectants. Several clones specific for CCR7 transfectants were derived, and a few of them, including 7H12 (murine IgG2b) and 3D9 (murine IgM), recognized a proportion of PBLs nearly identical with the proportion capable of responding to known CCR7 ligands. These two mAbs gave essentially identical staining patterns on all lymphocyte subsets tested, and only these clones were used for the studies reported here. The staining patterns of these two novel mAbs were very similar to that of the CCR7 mAb used in Ref. 9 on T cells. In further support of the specificity of these mAbs for CCR7, the ability to stain PBLs with the 7H12 and 3D9 mAbs was lost after treatment with the CCR7 ligand macrophage-inflammatory protein (MIP)-3β, but not after treatment with the CXCR4 ligand normal cell-derived factor (SDF)-1α (J.J.C., unpublished data). Thus, MIP-3β may either directly block binding of the mAbs to their cell surface epitope or cause internalization of their cell surface epitope (directly or indirectly). Indirect triggering of internalization seems unlikely, as in the reciprocal experiment pretreatment of PBL with the CXCR4 ligand SDF-1α abolished binding of two of commercially available anti-CXCR4 mAbs without affecting the binding of our CCR7 mAbs (J.J.C., unpublished data).

Four-color flow cytometry

Four-color flow cytometry was performed as in Ref. 2. mAbs were either directly conjugated to FITC, PE, or APC, or visualized by biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) followed by streptavidin-peridinin chlorophyll protein (Becton Dickinson, San Jose, CA) and then photographed. These two mAbs gave essentially identical staining patterns on all lymphocyte subsets tested, and only these clones were used for the studies reported here. The staining patterns of these two novel mAbs were very similar to that of the CCR7 mAb used in Ref. 9 on T cells. In further support of the specificity of these mAbs for CCR7, the ability to stain PBLs with the 7H12 and 3D9 mAbs was lost after treatment with the CCR7 ligand macrophage-inflammatory protein (MIP)-3β, but not after treatment with the CXCR4 ligand normal cell-derived factor (SDF)-1α (J.J.C., unpublished data). Thus, MIP-3β may either directly block binding of the mAbs to their cell surface epitope or cause internalization of their cell surface epitope (directly or indirectly). Indirect triggering of internalization seems unlikely, as in the reciprocal experiment pretreatment of PBL with the CXCR4 ligand SDF-1α abolished binding of two of commercially available anti-CXCR4 mAbs without affecting the binding of our CCR7 mAbs (J.J.C., unpublished data).

**Chemotaxis**

All migration assays were performed in Costar 24-well plate tissue-culture inserts with 5-μm pore size strainer filters. Cells (5 × 10⁷) were placed in the upper chamber in 100 μl, and 600 μl of a given chemokine dilution was placed in the lower well. Migration was carried out in migration medium (RPMI 1640/0.5% BSA fraction V; Sigma, St. Louis, MO). Migrated cells were counted as described previously (2, 11,–14). Migrating cells were kept at 37°C with 8% CO₂.

MIP-3β was synthesized by one of us (D.S.) or purchased in recombinant from Pharmacia Biotech (Rocky Hill, NJ). Both synthetic and recombinant forms behaved identically in parallel experiments. Recombinant secondary lymphoid-tissue chemokine (SLC) was purchased from PeproTech. Synthetic SDF-1α was purchased from Gryphon Sciences (South San Francisco, CA). Recombinant Burkit’s lymphoma receptor-1 ligand chemokine (BCL-1) was purchased from R&D Systems (Minneapolis, MN).

**Human peripheral blood and tissue lymphocytes**

Human peripheral blood was collected in heparinized tubes from healthy donors. Granulocytes were removed as described (15). Monocytes were removed by two 30-min rounds of adherence to a T-175 culture flask (Nunc, Roskilde, Denmark) at 37°C and 8% CO₂ in RPMI 1642 medium supplemented with 10% calf serum. Fresh tonsils were obtained after routine adenoid and tonsillectomy procedures. Tonsil lymphocytes were dispersed through stainless steel mesh and incubated in the same way as PBL before being lightly sedimented with midazolam, and the upper airway was anesthetized with 2% lidocaine. Normal saline (180 ml) was inserted through the bronchoscope into the right lower lobe and aspirated using gentle suction. Recovery was between 20 and 46%.

Lymphocytes from the lamina propria of human jejunal were isolated as described previously (16) with slight modification (i.e., the DTT step to degrade mucous was eliminated because we found that DTT destroyed the epitopes recognized by the anti-αβ, mAb ACT-1). Lymphocyte yield was not reduced by eliminating this step (data not shown). Lymphocytes were isolated from normal human skin by first using a razor blade to separate the epidermis and a portion of the dermis from the lower dermis and s.c. fat. The epidermal pieces were cut into strips and incubated in cold 5 mM EDTA/HBSS for 120 min with vigorous stirring. The supernatant from this step was spun down to obtain released lymphocytes, and the remaining strips were crushed through a 50-μm pore size strainer to obtain additional lymphocytes. Lymphocytes were isolated from normal lung by finely mincing the tissue and sieving the resulting suspension through gauze. Lymphocytes were isolated from explant livers by first cutting the liver into small 1-cm² pieces, washing in RPMI 1640 supplemented with 10% FBS, then homogenizing into a cell suspension with a Stomacher 400 (Seward, London, U.K.). Lymphocytes were isolated from synovial tissue by mincing and treating with EDTA as described above for epidermis. Synovial fluid and bronchoalveolar lavage fluid were diluted with FACS buffer (PBS supplemented with 0.1% azide and 1% BSA), centrifuged at 250 × g, and resuspended in FACS buffer. Cell suspensions from liver were layered over Ficoll (Amersham Pharmacia Biotech, Piscataway, NJ), and the mononuclear cell layer was used for FACS analysis. We found that the treatments required to dissociate these tissues did not affect CCR7 expression, and other lymphocyte markers such as LFA-1 were positive on all isolated lymphocyte populations (data not shown).

**Results**

Functional expression of CCR7 on tissue-specific memory CD4 cells from peripheral blood

We tested the hypothesis (9, 17) that CCR7 expression (essential for homing of naive T-cells to secondary lymphoid tissues; Refs. 5–7) is lost in memory T cells that express adhesion receptors necessary for homing to nonlymphoid tissues. We generated two novel mAbs to human CCR7 (7H12 and 3D9, see Materials and Methods).

The best studied peripheral blood memory CD4 T cells dedicated to specific nonlymphoid tissues are the skin-homing cells (CLA(pos)/αβ, neg) and the gut-homing cells (CLA(neg)/αβ, neg) (reviewed in Ref. 1). Four-color flow cytometry was used to examine CCR7 expression on these two populations, as well as on naive CD4 cells and CLA(neg)/αβ, neg (double negative memory CD4 cells of unknown tissue-homing specificity (Fig. 1A). Naive and double negative memory CD4 cells were nearly 100% positive for CCR7 expression (Fig. 1B). Skin- and gut-homing memory CD4 cells were somewhat enriched in CCR7 cells (Fig. 1B), but the vast majority of both populations were CCR7(pos). A survey of peripheral blood CD4 subsets from 10 healthy donors (Fig. 1C) shows that skin- and gut-homing populations were ~80% CCR7(pos), whereas naive and double negative memory cells were nearly 100% CCR7(pos).

Migration of these four peripheral blood CD4 populations to known CCR7 ligands (MIP-3β and SLC) was similar to their migration to SDF-1α, a nearly universal lymphocyte chemotactic receptor (11, 18) (Fig. 1D). Thus, the vast majority of naive, memory, and tissue-dedicated CD4 cells from peripheral blood express CCR7, respond to CCR7 ligands, and can probably home to secondary lymphoid organs.

**CCR7 vs other markers on peripheral blood CD4 cells**

As CCR7 loss correlated only poorly with expression of tissue-dedicated homing receptors, we set out to identify other features of CCR7(neg) CD4 T subsets. Fig. 2 shows flow cytometry of CCR7 vs several other markers on CD4(pos) T cells from peripheral blood. As expected from Fig. 1, the CCR7(neg) population contained subsets of CLA(pos) and of αβ, high cells (Fig. 2, top). All CCR7(neg) cells were contained within the CD45RA(neg) memory population (Fig. 2, second row left). Most CCR7(neg) cells

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This text is a sample from a larger scientific document. The document contains detailed experiments, results, and discussions related to the expression and function of CCR7 in human lymphocytes. It highlights the importance of CCR7 in the migration and homing of immune cells to various tissues and demonstrates the specificity of anti-CCR7 antibodies in identifying these cells. The methodologies used include flow cytometry for the analysis of cellular subsets and chemotaxis assays to evaluate cell migration in response to specific chemokines.
lacked L-selectin, consistent with the notion that these cells are incapable of homing to secondary lymphoid organs (Fig. 2, third row left). Interestingly, CCR7(neg) cells were nearly 100% positive for CCR5, the receptor for MIP-1β, RANTES, and several other CC chemokines (the anti-CCR5 mAb 2D7 was used for these studies because it was shown to correlate best with genetic expression of CCR5 when compared with other CCR5 mAbs; Ref. 19) (Fig. 2, third row right). Interestingly, CCR7(neg) cells were also enriched in CD11b (MAC-1), a β2 integrin commonly expressed in the myeloid lineages and B1 B cells found in serosal cavities, but rare on T lymphocytes (Fig. 2, second row right). CCR7(neg) cells often lacked CD27 (a member of the TNF-α receptor family; Fig. 2, bottom). Moreover, over 50% of the CD27(neg) CD4 cells also lacked CCR7.

The CD27(neg) subset of peripheral blood memory CD4 cells

The enrichment of CD27(neg) cells in the CCR7(neg) CD4 T cell compartment was of great interest to us because CD27 loss has been reported to mark both CD4 (20, 21) and CD8 (22) T cells of effector phenotype. As Sallusto et al. had reported that the CCR7(neg) CD4 population is enriched in an effector-like phenotype, we set out to more fully define the relationship between CCR7 and CD27 expression on peripheral blood CD4 cells. Fig. 3A shows that staining of CD4 cells with CD45RA and CD27 cleanly divides them into three distinct subsets: CD45RA(pos)/CD27(pos) naive cells; CD45RA(neg)/CD27(pos) conventional memory cells; and CD27(neg) memory cells. Expression of CCR7, CCR5 (2D7), MAC-1, and L-selectin was examined individually on each of these populations (Fig. 3B). Naive cells all expressed both L-selectin and CCR7, consistent for their known tropism for secondary lymphoid tissues (Fig. 3A, top). Conventional memory cells contained a subpopulation of CCR7(neg) and L-selectin(neg) cells, but the vast majority was positive for both. Some conventional memory cells expressed CCR5, and a very small number expressed MAC-1 (Fig. 3A, middle). The CD27(neg) memory population was very different from the naive and conventional memory subsets (Fig. 3A, bottom). Most CD27(neg) memory cells expressed neither CCR7 nor L-selectin. Many of these cells expressed CCR5 and MAC-1, markers that were never seen on naive cells. A survey of 10 healthy donors showed that CCR7(neg) cells were quite rare (as a percentage) in the naive and conventional memory subpopulations, but common within the CD27(neg) memory population (Fig. 3C). Responsiveness to known CCR7 ligands in chemotaxis assays correlated with CCR7 expression (Fig. 3D). Most naive and conventional memory cells responded well to CCR7 ligands. Less than half of the CD27(neg) cells responded to CCR7 ligands. All three populations respond equally well to SDF-1α (Fig. 3D, right).

Diversity of memory CD4 cells lacking CCR7, CD27, or L-selectin

The above analysis of CD27(neg) memory cells shows them to be a population greatly enriched in cells of unusual (presumptive lymphoid tissue-excluded) phenotype. However, this observation is

FIGURE 2. CCR7 vs other markers on peripheral blood CD4 cells. Flow cytometry of CD4-gated PBLs showing CCR7 staining (y-axis) vs either CLA, α4β7 integrin, CD45RA, MAC-1, L-selectin, CCR5, or CD27 (x-axis). All plots are from a single donor. Representative of full profiles on two other donors and partial profiles on 12 donors. See Materials and Methods for mAb clones used.
only part of the story, and may be misleading. The relationship between CCR7 and CD27 is only an enrichment, not a direct correlation. In fact, if we stain peripheral blood CD4 cells for CCR7, CD27, and L-selectin together (Fig. 4), we see that by gating on either CCR7(neg), CD27(neg), or L-selectin(neg) cells, we enrich in cells that are lacking either or both of the other two markers. Thus, the message from these studies is that neither CCR7, CD27, nor L-selectin define a distinct population. There is extreme diversity in the cells lacking any one of these markers, whereas there is relative uniformity among the majority of both memory and naive lymphocytes, which express all three of these markers together.

CCR7 expression on peripheral blood CD8(pos) T cell subsets

We next examined the CCR7 expression of peripheral blood CD8 T cell subsets. As mentioned above, CD45RA and CD27 can be used to identify naive, memory, and effector CD8 subsets (22) (Fig. 5A). CCR7 expression and responsiveness to CCR7 ligands was tested on each of these peripheral blood CD8 populations. Like naive CD4 cells, naive CD8 cells (CD45RA(pos)/CD27(pos)) all expressed CCR7 and responded well to MIP-3β and SLC (Fig. 5, B and C). The memory CD8 population contained more CCR7(neg) than conventional memory CD4 cells (~50%), which correlated well with their lower responsiveness to CCR7 ligands.

Effector CD8 cells were nearly all negative for CCR7 expression and did not respond to CCR7 ligands. All three peripheral blood CD8 subsets responded equally well to SDF-1α (Fig. 5C, right).

CCR7 expression in tonsil T cell populations

As a representative secondary lymphoid organ for humans, tonsillar T cells were examined for CCR7 expression. As in peripheral blood, tonsil T cells can be divided into naive and memory based on CD45RA expression. However, unlike peripheral blood, most CD45RA(neg) T cells from the tonsil expressed the early activation marker CD69, suggesting that they were in an activated state (Figs. 6A and 7A). Most CD45RA(pos) naive tonsil T cells did not express CD69 (Figs. 6A and 7A).

Tonsil CD4 cells. Chemokine receptor expression was examined separately on naive (CD45RA(pos)/CD69(neg)) and activated (CD45RA(neg)/CD69(pos)) tonsil cells. Like naive CD4 cells from the circulation, essentially all naive tonsil CD4 cells expressed high levels of CCR7. This is consistent with the notion that CD4 cells require CCR7 for homing to secondary lymphoid tissues through HEV. The tonsil contained almost no CD27(neg) cells (Fig. 7), which may be unable to enter secondary lymphoid organs because of their low CCR7 and L-selectin levels.

The presence or absence of CCR7 has been implicated in determining microenvironmental movements of activated T cells within secondary lymphoid organs of mouse (23). Therefore, it was of great interest to compare CCR7 expression between naive and activated cells in this organ. Unlike naive cells, activated cells were markedly diverse in their CCR7 expression (Fig. 6B). The activated CD4 T cells express CCR7, on average, at lower levels than naive cells, and there was a large population of CCR7(neg) cells. Responsiveness to CCR7 ligands again parallels CCR7 expression between naive and activated cells (Fig. 6C). Another chemokine receptor, CXCR5 (BLR-1/EBI-2) has also been implicated in microenvironmental T cell movements (24). Expression of CXCR5, originally cloned as a B cell chemokine receptor, may allow activated T cells to migrate into B cell zones to provide T cell help (25). We found that expression of CXCR5 was nearly

FIGURE 3. Comparison of CD45RA(neg)/CD27(neg) peripheral blood CD4 cells to conventional naive and memory CD4 cells. A, Four-color flow cytometry dividing peripheral blood CD4 cells into naive (population 1), conventional memory (population 2), and CD27(neg) memory (population 3). B, Flow cytometry of each population above with anti-CCR7, CCR5, MAC-1, or L-selectin (solid lines); overlaid on isotype controls (dotted lines). C, Bar graph showing proportion of cells within each population expressing CCR7 with 7H12. Mean and SD for 10 donors. D, Chemotaxis of each population to titrations of CCR7 ligands and SDF-1α. Mean and range from two donors are shown, representative of titrations on four donors and nine donors tested at only the optimal concentrations.

FIGURE 4. Cells lacking CCR7, CD27, or L-selectin form an extremely heterogeneous network of mutually overlapping populations. PBLs were stained simultaneously with anti-CD4, CCR7, CD27, and L-selectin mAbs. All plots are from the same donor on the same day, shown with various gating criteria. The figure is representative of the same experiment performed on two other donors.

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opposite to that of CCR7. Naive tonsil cells expressed no CXCR5, whereas most activated cells expressed CXCR5. In agreement with this, activated but not naive cells migrated to the CXCR5 ligand BLC/BCA-1 (Fig. 6C). Both naive and activated CD4 cells in the tonsil responded equally well to SDF-1α (Fig. 5C, right).

**Tonsil CD8 cells.** Like CD4 cells, tonsil CD8 cells could be cleanly subdivided into CD45RA(pos)/CD69(neg) naive and CD45RA(neg)/CD69(pos) activated subsets (Fig. 7A). However, naive CD8 cells were much more abundant than activated CD8 cells, unlike CD4 cells in which the naive and activated populations were nearly equal (Fig. 4A). This is consistent with the finding (Fig. 5) that a large proportion of memory CD8 cells from peripheral blood do not express CCR7 (and CD8 effector cells from blood are nearly devoid of CCR7) and thus may be unable to home to secondary lymphoid organs. CCR7 and CXCR5 expression is similar to that of the homologous CD4 populations. Naive cells express uniformly high levels of CCR7 and no CXCR5. Activated CD8 cells are heterogeneous for CCR7 expression and express CXCR5 (Fig. 7B). Responsiveness to chemokines again parallels receptor expression (Fig. 7C).

**CCR7 expression in T lymphocytes infiltrating nonlymphoid tissues**

To further understand the role of CCR7 in routine lymphocyte trafficking, immunosurveillance, and homing to inflamed sites, CCR7 expression was examined on tissue-infiltrating lymphocytes freshly isolated from various nonlymphoid tissues. CD8 cells were rare in most tissues examined, so our study focused on CD4 cells only. Contamination of tissue-infiltrating lymphocytes with peripheral blood cells was not significant, as naive cells, which constitute nearly 50% of peripheral blood CD4 cells, were consistently absent from tissue populations (see Figs. 1 and 3A).

Unlike CD45RA(neg) T cells from secondary lymphoid tissue (tonsil), CD27(neg) cells were quite common in nonlymphoid tissues. The ratio of CD27(neg) to CD27(pos) cells varied greatly from tissue to tissue (Fig. 8) but was similar among donors for a given tissue (data not shown). CD27(neg) cells from all tissues were consistently CCR7(neg). The CD27(pos) populations contained both CCR7(neg) and CCR7(pos) subsets.

CD4 lymphocytes isolated from small intestine (jejunum) lamina propria (n = 3), liver (n = 3), dispersed lung tissue (n = 4), and the bronchial space of lung (n = 4) were consistently CD27(neg)/CCR7(neg) (Fig. 8). Those isolated from normal skin (from face lifts, n = 3) and from synovium or synovial fluid of autoimmune arthritis patients (n = 1 and 4, respectively) contained many more CD27(neg)/CCR7(neg) than patient-matched blood (data not shown), but the majority of infiltrating cells from these tissues were CD27(pos)/CCR7(pos) (Fig. 8).

**Discussion**

Peripheral blood T cells lacking CCR7

CCR7 expression is not lost upon differentiation into skin- or gut-homing phenotypes. We have examined the expression and function of CCR7 in lymphocytes from peripheral blood, secondary lymphoid tissues, and nonlymphoid tissues. An existing hypothesis suggests that for a memory T cell to differentiate into a form capable of homing to nonlymphoid tissues, it must lose the ability (possessed by all naive T cells) to home to secondary lymphoid tissue via HEV (9, 17).

We have found that, like naive and most other memory T cells, the majority of tissue-specific memory T cells (i.e., skin- and gut-homing) still expressed CCR7, and are thus (because they also express L-selectin and LFA-1; Ref. 26) likely still competent to enter secondary lymphoid tissues. Skin- and gut-homing populations are moderately enriched in CCR7(neg) cells, indicating that loss of CCR7 may occur after differentiation into tissue-specific...
phenotypes or that these two differentiation processes may be entirely independent.

**CCR7-negative peripheral blood CD4 cells.** CCR7 was missing from only a minor subset of peripheral blood memory CD4 T cells. All naive T cells, and >90% of memory CD4 T cells (defined by CD45RA phenotype) expressed high levels of CCR7. The CCR7(pos) memory cells were relatively uniform in expression of L-selectin and CD27, like the naive population (although, of course, they aren’t uniform in homing receptor expression). Enrichment of CCR7(neg) cells in the CD27(neg) population was intriguing, and was examined in detail.

Memory T cells lacking CD27 have been previously associated with effector properties (20–22). We found that besides being enriched in CCR7(neg) cells, the CD27(neg) population of CD4 T cells contained an intermediate level of CCR7-expressing cells. However, again, CD8 cells of effector phenotype could also consist of recently activated cells that have not yet regained the ability to traffic through lymphoid organs.

**CCR7 in lymphocyte homeostasis and immunosurveillance**

There have been three (nonexclusive) hypotheses for the roles of CCR7 in lymphocyte homing to date: 1) recognition of SLC presented by HEV of secondary lymphoid tissues, triggering arrest of circulating T cells on endothelium as a prerequisite to migration into the tissue. In this role, CCR7 would be an HEV homing receptor, along with L-selectin and LFA-1. There is now ample support for this hypothesis in animal models (5, 6; reviewed in Ref. 4);
FIGURE 8. Expression of CCR7 on CD27(neg) and CD27(pos) tissue-infiltrating CD4 T cells. CD27 vs CCR7 staining of CD4(pos)/CD45RA(neg)-gated tissue-infiltrating lymphocytes. Lymphocytes were isolated from tissues using gentle mechanical agitation and chelation, without enzymatic digestion (see Materials and Methods). Representative dot-plots for each organ type shown. Three jejunal lamina propria preparations (from gastric bypass operations) were tested, four bronchoalveolar lavages (from nonasthmatic donors), three dispersed lung-tissue, three dispersed lung-tissue specimens, three cirrhotic livers (two alcoholic and one hepatitis C), three normal skin preparations (from face-lifts), four synovial fluid samples (two from RA and two from psoriatic arthritis), one RA synovium, and five tonsils (from routine adenoid and tonsillectomies).

2) migration through gradients of MIP-3β expressed within secondary lymphoid organs, which coordinates proper microenvironmental localization within the organ. This hypothesis also has support in animal models, as manipulation of CCR7 expression can alter the microanatomic location of T cells within the spleen (23); 3) migration of tissue-infiltrating lymphocytes back into the lymphatic system. This has been proposed because endothelial cells lining the lymph vessels have been found to express mRNA for CCR7 ligands (8). This hypothesis has not yet been tested for lymphocytes in experimental models, but is supported by studies of DC migration (27–31). We have examined CCR7 expression on T cells derived from each of the locations where it is thought to have influence (peripheral blood (discussed above), secondary lymphoid tissue, and nonlymphoid tissue) to shed light on these hypotheses.

Tonsil T cells. Secondary lymphoid tissues are very high-traffic zones for lymphocytes. T and B cells are constantly entering from the blood through HEV. Most cells entering lymphoid organs do not encounter their cognate Ag, and pass through the organ to return to the circulation via afferent lymph. However, those that do encounter Ag and become activated must be directed to microenvironmental locations where they can perform their helper or effector functions. It has been proposed that modulation of chemokine responses may mediate proper microenvironmental localization (25). We have found that CCR7 is expressed at high levels on naive T cells (both CD4 and CD8) within the tonsil, comparable to that of circulating naive cells. In contrast, many cells activated in the lymphoid organ may lose CCR7, which may allow them to enter microenvironments not normally entered by unactivated cells. Consistent with this notion, activated T cells gain the expression of CXCR5 (and perhaps other chemokine receptors not expressed by resting or naive cells), thus gaining the ability to respond to new chemotactic gradients not perceived by other cells. It is interesting that both CD4 and CD8 T cells undergo very similar changes in CCR7 and CXCR5 expression after activation; this strongly implies the participation of other chemokines in fine-tuning the microenvironmental localization of functionally distinct lymphocytes, as modeled in Refs. 32 and 33. Our findings are consistent with the hypothesis that chemokine responsiveness patterns are actively altered in different subsets of lymphocytes, a mechanism that may orchestrate the complex interplay among APCs, T cells, and B cells within lymphoid organs.

Nonlymphoid tissues. Although naive T lymphocytes can be found almost exclusively in the blood and lymphoid organs, memory T cells can be found at low levels in every tissue of the body (34). We have examined the CCR7 expression of CD4 T cells isolated from several types of nonlymphoid tissues. One important finding from this work is that many tissue-infiltrating lymphocytes express CCR7. This further argues against the hypothesis that CCR7-expressing cells do not infiltrate nonlymphoid tissues (9, 17).

We have found a correlation between CCR7 and CD27 expression in tissue-infiltrating lymphocytes, which parallels our findings in peripheral blood. CD27 is a member of the TNF-α receptor family, and its modulation may mark previously unknown activation or developmental states in tissue-infiltrating lymphocytes. However, loss of CD27 expression does not correlate well with expression of the activation marker CD69, which is was generally expressed on all tissue-infiltrating lymphocytes studied (data not shown).

Nearly all CD27(neg) tissue-infiltrating lymphocytes lacked CCR7 expression (Fig. 8). Lymphocytes from normal intestinal tissue, normal lung, and cirrhotic liver are nearly all CD27(neg)/CCR7(neg). Lymphocytes from normal skin and from synovium or synovial fluid of rheumatoid arthritis-afflicted joints are more heterogeneous and contain many CD27(pos)/CCR7(pos) cells. Normal skin (with mostly CD27(pos)/CCR7(pos) memory cell infiltrates) and gut (with mostly CD27(neg)/CCR7(neg) memory infiltrates) are sites with very different levels of immunological activity. The gut tissue of normal individuals is thought to be constantly involved in immune reactions to food Ags and intestinal flora. This has led to the suggestion that intestinal tissue be considered a tertiary lymphoid organ (reviewed in Ref. 34). In contrast, skin presents a barrier to the outside world and would only engage in inflammatory reactions when the barrier is broken. Thus, normal skin would not be expected to contain much immune activity.

The patterns of CCR7 expression within these two (very different) tissues fits well with the notion that CCR7 ligands may direct tissue-infiltrating T cells back to the lymph (8). Based on the known levels of immune activity in these organs, CD27(neg) cells may represent a population that is actively engaged in Ag recognition. Therefore, this population is rare in skin but common in intestine. It would not be desirable for such actively engaged cells to return to the lymph; thus, these cells do not express CCR7. However, in the skin, where Ag recognition is not common, most lymphocytes are passing through the tissue in routine immunosurveillance, are not actively engaged in Ag recognition, and continue to express CD27. Such cells, not encountering Ag, continue to
express CCR7 to guide them back to the lymph, completing their circuit through the tissue.

The arguments used above for gut-infiltrating lymphocytes would also apply to lung and cirrhotic liver, whose infiltrating lymphocytes are phenotypically similar to gut (Fig. 8). The high CCR7 expression in rheumatoid synovium and synovial fluid requires another explanation: perhaps an autoimmune chronically inflamed situation may greatly enhance the amount of lymphocyte trafficking through the inflamed tissue (with respect to its uninflamed counterpart). Only a small proportion of cells passing through such a high-traffic area would be specific for the autoantigen. Thus, just like normal skin, most cells infiltrating rheumatoid arthritis synovium may not see Ag, and continue to express CD27 and CCR7. Alternatively, the number of active cells in synovium and synovial fluid samples could be lower due to anti-inflammatory treatments the donors may have received.

Conclusions. In this study we 1) analyzed in detail the population of CCR7(neg) cells, which have been the focus of great interest recently; and 2) investigated the expression of CCR7 on cells at various points in the cycle of routine immunosurveillance in an attempt to shed light on the role of CCR7 and its ligands in these processes. We found that CCR7, CD27, and L-selectin, mark an intensely heterogeneous series of overlapping populations. No single one of these markers can be used to identify a uniform population with respect to the others. It is likely that a combination of these markers is required to identify all true effector memory cells.

We also found that the CCR7 expression patterns on T cells are consistent with known or hypothesized roles for each of the three major nexus points of immunosurveillance: trafficking into secondary lymphoid organs, movement and organization within lymphoid organs, and trafficking out of nonlymphoid organs and into the lymph.

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References
