

## CUTTING EDGE

Cutting Edge: Expression of Chemokine Receptor CXCR1 on Human Effector CD8<sup>+</sup> T Cells<sup>1</sup>Hiroshi Takata, Hiroko Tomiyama, Mamoru Fujiwara, Naoki Kobayashi, and Masafumi Takiguchi<sup>2</sup>

*IL-8 is a potent inflammatory cytokine that induces chemotaxis of neutrophils expressing CXCR1 and CXCR2, thus indicating its involvement in the migration of these cells to inflammatory sites where bacteria proliferate. Presently, we showed that CXCR1<sup>+</sup> cells were predominantly found among CD8<sup>+</sup> T cells having effector phenotype, and that the expression of CXCR1 was positively correlated with that of perforin, suggesting that CXCR1 is expressed on effector CD8<sup>+</sup> T cells. Indeed, human CMV-specific CD8<sup>+</sup> T cells from healthy individuals, which mostly express the effector phenotype and have cytolytic function, expressed CXCR1, whereas EBV-specific CD8<sup>+</sup> T cells, which mostly express the memory phenotype and have no cytolytic function, did not express this receptor. The results of a chemotaxis assay showed that the migration of CXCR1<sup>+</sup>CD8<sup>+</sup> T cells was induced by IL-8. These results suggest that the IL-8-CXCR1 pathway plays an important role in the homing of effector CD8<sup>+</sup> T cells. The Journal of Immunology, 2004, 173: 2231–2235.*

Memory and effector CD8<sup>+</sup> T cells play an important role in the eradication of viruses and tumor cells through their ability to produce various factors involved in the suppression of viral replication (1, 2) and to cause cytolysis of virus-infected and tumor cells (3). Effector CD8<sup>+</sup> T cells have the ability to kill target cells through the action of perforin and Fas ligands. These cells express a high level of perforin and produce cytokines such as TNF- $\alpha$  and IFN- $\gamma$  (4). Previous studies suggested that particular expression patterns of costimulatory receptors CD27 and CD28 as well as CD45RA or CD45RO are associated with the naive, memory, and effector functions of human CD8<sup>+</sup> T cells (5–8). Multicolor flow cytometric analysis demonstrated that effector and memory/effector CD8<sup>+</sup> T cells, both of which have cytolytic activity, have the phenotypes of CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> and CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup>, respectively (4).

The chemokine receptor CCR7 is useful for discriminating naive and central memory CD8<sup>+</sup> T cells from memory/effector and effector CD8<sup>+</sup> T cells (9). CCR7 functions as a homing

receptor and is expressed on naive CD8<sup>+</sup> T cells and on a subset of memory CD8<sup>+</sup> T cells. A previous study resulted in the following classification of CD8<sup>+</sup> T cells based on CCR7 and CD45RA: naive, CCR7<sup>+</sup>CD45RA<sup>+</sup>; central memory, CCR7<sup>+</sup>CD45RA<sup>-</sup>; and effector/memory, CCR7<sup>-</sup>CD45RA<sup>+/-</sup> (9). A recent study showed that the CCR5 chemokine receptor is expressed on memory, memory/effector, and effector CD8<sup>+</sup> T cells, with the number of CCR5<sup>+</sup>CD8<sup>+</sup> T cells decreasing during the differentiation of CD27<sup>+</sup>CD28<sup>+</sup>CD45RA<sup>-</sup> memory T cells into CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> effector T cells (5). These findings imply that these two chemokine receptors are also useful for the classification of CD8<sup>+</sup> T cells.

Two IL-8 receptors, CXCR1 and CXCR2, are expressed on the surface of neutrophils, monocytes, mast cells, NK cells, and eosinophils (10–12). IL-8 can attract these cells (11), indicating that these IL-8 receptors play an important role in the migration of these cells to sites of inflammation. Thus, the cells expressing these IL-8 receptors are involved in innate immunity. Concerning the cells involved in acquired immunity, such as CD8<sup>+</sup> and CD4<sup>+</sup> T cells, a previous study showed that some CD8<sup>+</sup> T cells express these receptors (13). However, there has been no detailed analysis regarding the expression of these receptors on CD8<sup>+</sup> T cells.

In the present study, we investigated the expression and function of CXCR1 on human CD8<sup>+</sup> T cells. The results showed that CXCR1 was expressed on effector CD8<sup>+</sup> T cells and that the migration of CXCR1<sup>+</sup>CD8<sup>+</sup> T cells was induced by IL-8. Our present data have thus revealed an important role played by CXCR1 in acquired immunity.

## Materials and Methods

## Antibodies

Anti-CD27-FITC, anti-CXCR1-FITC (5A12), anti-CD28-allophycocyanin, and anti-perforin-FITC mAbs were obtained from BD Pharmingen (San Diego, CA). Anti-CD45RA-ECD was purchased from Immunotech (Marseille, France). Anti-CD3-FITC, anti-CD8-allophycocyanin, anti-CXCR1-PE (42705.111), and anti-CXCR2-PE (48311.211) mAbs were obtained from DakoCytomation (Glostrup, Denmark). Human IgG came from Sigma-Aldrich (St. Louis, MO).

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### HLA-class I tetramers

HLA-class I-peptide tetrameric complexes (tetramers) were synthesized as previously described (14). The human CMV (HCMV)<sup>3</sup> CTL epitope (HCMV-1 pp65<sub>495-503</sub>; NLVPMVATV; Ref. 15) and the EBV CTL epitope (EBV 3B 399–408, AVFDRKSDAK; Ref. 16) were used for refolding of HLA-A\*0201 or HLA-A\*0206 molecules and HLA-A\*1101 molecules, respectively. PE-labeled streptavidin (Molecular Probes, Eugene, OR) or allophycocyanin-Cy7-labeled streptavidin (Cedarlane Laboratories, Hornby, Ontario, Canada) was used for generation of tetramers.

### Flow cytometric analysis

PBMCs from healthy individuals were stained with anti-CD3 mAb, anti-CD8 mAb, and anti-CXCR1 mAb or anti-CXCR2 mAb. The cells were incubated at 4°C for 30 min, and were then washed two times with PBS containing 10% newborn cow serum (PBS/10% NCS). The percentage of CXCR1<sup>+</sup>CD8<sup>+</sup> T cells in the total CD8<sup>+</sup> T cells was determined by using a FACSCalibur (BD Biosciences, San Jose, CA). Furthermore, to clarify CXCR1 expression in each CD27CD28CD45RA subset of total CD8<sup>+</sup> T cells, we purified CD8<sup>+</sup> T cells from PBMC by using anti-CD8-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). The percentage of CD8<sup>+</sup> T cells among the purified cells was >98%. Purified CD8<sup>+</sup> T cells were stained with anti-CD27 mAb, anti-CD28 mAb, anti-CD45RA mAb, and anti-CXCR1 mAb. The cells were incubated at 4°C for 30 min, and were then washed two times with PBS/10% NCS. The percentage of CXCR1<sup>+</sup> cells in each subset was measured by using the FACSCalibur.

To define intracellular perforin expression in CXCR1<sup>+</sup> and CXCR1<sup>-</sup> subsets of total CD8<sup>+</sup> T cells, we stained PBMCs with anti-CD8 mAb and anti-CXCR1 mAb, fixed them in 4% paraformaldehyde at 4°C for 20 min, and then permeabilized them at 4°C for 10 min with PBS containing with 0.1% saponin and 20% NCS (permeabilizing buffer). The cells were washed with permeabilizing buffer and then resuspended in 50  $\mu$ l of the same buffer. After anti-perforin mAb had been added, the cell suspension was incubated at 4°C for 30 min, and the cells were then washed three times in the permeabilizing buffer at 4°C. PE-labeled mouse IgG1 was used as a negative control.

To determine the expression of CXCR1 on HCMV-specific and EBV-specific CD8<sup>+</sup> T cells, we purified CD8<sup>+</sup> T cells and mixed them with HCMV-1-A\*0201, HCMV-1-A\*0206 or EBV-1-A\*1101 tetramers at a concentration of 0.02–0.04 mg/ml. After incubation at 37°C for 30 min, the cells were washed two times with RPMI 1640/10% NCS, and then anti-CXCR1 mAb only or anti-CD27 mAb, anti-CD28 mAb, anti-CD45RA mAb, and anti-CXCR1 mAb were added to the cell suspension. The cells were incubated at 4°C for 30 min, and were then washed two times with PBS/10% NCS.

### Assay for cytotoxic activity

Cytotoxic activity was measured by the standard <sup>51</sup>Cr-release assay as follows. Target cells ( $2 \times 10^5$ ) were incubated for 60 min with 100  $\mu$ Ci Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub> in saline and washed three times with RPMI 1640/10% NCS. Labeled target cells ( $2 \times 10^3$ /well) were added to U-bottom 96-well plates with the corresponding peptide ( $10^{-6}$  M). After 1 h incubation, CD8<sup>+</sup> T cells purified by anti-CD8 mAb-coated beads or CXCR1<sup>+</sup>CD8<sup>+</sup> T cells purified by a cell sorter (FACSARIA; BD Biosciences) were added, and the mixtures were then incubated for 6 h at 37°C. The supernatants were then collected and analyzed with a gamma counter.

### Lymphocyte chemotaxis assay

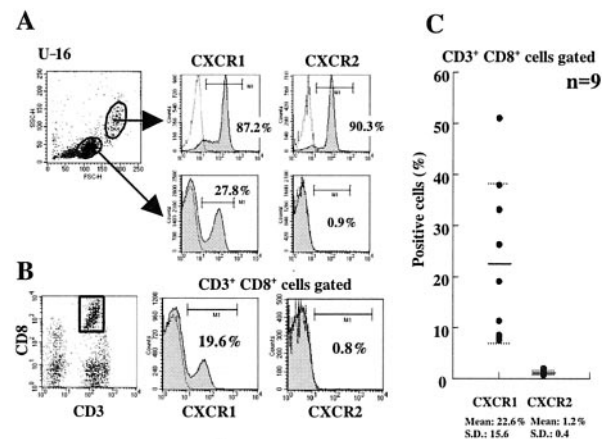
The lymphocyte chemotaxis assay was performed by using 96-well microchemotaxis chambers (NeuroProbe, Gaithersburg, MD) as follows. The CD8<sup>+</sup> T cells or CXCR1<sup>-</sup>CD8<sup>+</sup> T cells purified by a cell sorter ( $5 \times 10^5$  cells/well) were placed over the filter (5- $\mu$ m pore diameter) in the upper wells of the chamber. Recombinant human IL-8 (BD Biosciences) was diluted with RPMI 1640 and then applied to the lower wells of the chamber. After incubation at 37°C for 3 h, the cells that had migrated through the filter were collected by centrifugation and counted. The data were expressed as the average number of cells that had migrated in three wells. The cells that had migrated nonspecifically were excluded.

## Results and Discussion

A previous study showed that IL-8 receptors CXCR1 and CXCR2 were expressed on the surface of some CD8<sup>+</sup> T cells

(13). To confirm this, we investigated the surface expression of CXCR1 and CXCR2 on total CD8<sup>+</sup> T cells. We first examined their surface expression on PBMC by using anti-CXCR1 and anti-CXCR2 mAbs. CXCR1<sup>+</sup> cells were detected among both small-size (lymphocytes) and large-size (monocytes) cells, whereas CXCR2<sup>+</sup> cells were found only among the latter (Fig. 1A). These results support a previous finding that both IL-8 receptors are expressed on monocytes (10), and suggest that only CXCR1 is expressed on T cells. To clarify the expression of these receptors on T cells, we examined the CXCR1 and CXCR2 expression on T cells among PBMCs from healthy individuals by using anti-CD3, anti-CD8, and anti-CXCR1 or anti-CXCR2 mAbs. A representative result is shown in Fig. 1B. A significant number of CD8<sup>+</sup> T cells expressed CXCR1, whereas almost no CD8<sup>+</sup> T cells expressed CXCR2 (Fig. 1B). In contrast, CD8<sup>-</sup> T cells did not express CXCR1 (data not shown). The lack of CXCR2 expression on CD8<sup>+</sup> T cells was confirmed by examining the cells from nine healthy individuals. The expression of CXCR1 varied among these nine individuals, with the percentage of CXCR1<sup>+</sup>CD8<sup>+</sup> T cells among the total CD8<sup>+</sup> T cells ranging from 7.6 to 51.0% (mean  $\pm$  SD, 22.6  $\pm$  15.6%; Fig. 1C). These results indicate that a given subset of CD8<sup>+</sup> T cells expresses CXCR1, but are in conflict with a previous finding that CD8<sup>+</sup> T cells express CXCR2 (10).

Our recent study showed that naive, memory, and effector CD8<sup>+</sup> T cell subsets can be classified by CD27, CD28, and CD45RA (4). Therefore, we investigated the surface expression of CXCR1 on each CD27CD28CD45RA subset in the total CD8<sup>+</sup> T cells. Total CD8<sup>+</sup> T cells were isolated from PBMC of 10 healthy individuals, and then analyzed by four-color flow cytometric analysis with anti-CD27, anti-CD28, anti-CD45RA, and anti-CXCR1 mAb. CXCR1-positive cells were found in subsets with CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> and



**FIGURE 1.** Surface expression of CXCR1 on CD8<sup>+</sup> T cells. *A*, Surface expression of CXCR1 and CXCR2 on PBMC. PBMCs from a given individual (U16) were stained with anti-CXCR1 or -CXCR2 mAb. Frequency of CXCR1<sup>+</sup> and of CXCR2<sup>+</sup> cells in both small- and large-size cell populations was analyzed by flow cytometry. The percentage of CXCR1<sup>+</sup> or CXCR2<sup>+</sup> cells is given in each plot. *B*, Surface expression of CXCR1 and CXCR2 on CD8<sup>+</sup> T cells. PBMC from individual U16 were stained with anti-CD3, anti-CD8, and anti-CXCR1 or anti-CXCR2 mAbs. The CD3<sup>+</sup>CD8<sup>+</sup> subset was gated, and then the surface expression of CXCR1 and of CXCR2 was analyzed. The percentage of CXCR1<sup>+</sup> cells or CXCR2<sup>+</sup> cells is given in each plot. *C*, Variation of CXCR1 and CXCR2 expression on CD8<sup>+</sup> T cells in nine healthy individuals. The mean percentage and SD of CXCR1<sup>+</sup> cells and CXCR2<sup>+</sup> cells in the CD3<sup>+</sup>CD8<sup>+</sup> subset are presented in the figures.

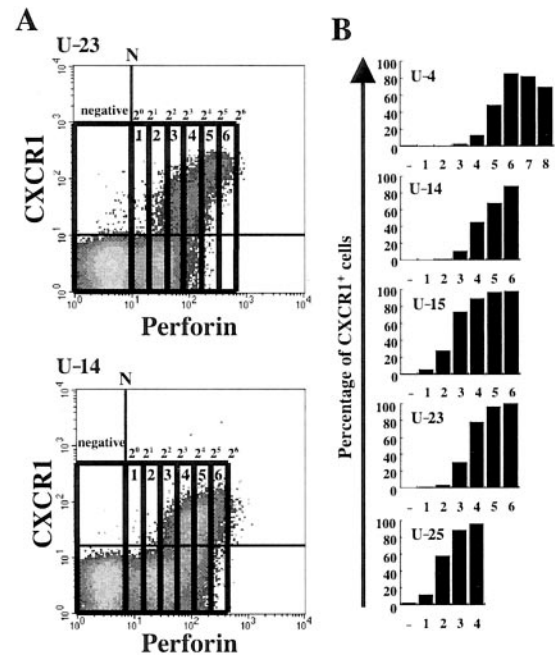
<sup>3</sup> Abbreviations used in this paper: HCMV, human CMV; GCP, granulocyte chemotactic protein; MFI, mean fluorescence intensity; NCS, newborn cow serum.

CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> phenotypes (Fig 2A). Approximately 60% of the CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> cells expressed CXCR1, whereas only 20% of CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> cells expressed this receptor (Fig. 2B). Because these subsets have cytotoxic activity (4, 6), these results suggest that CXCR1 is expressed only on the subsets with cytolytic effector function.

Because CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> subsets express a much higher level of perforin than other CD27CD28CD45RA subsets (4), we suspected that CXCR1 expression would be positively correlated with perforin expression in CD8<sup>+</sup> T cells. Therefore, we analyzed the correlation between CXCR1 and perforin expression on/in CD8<sup>+</sup> T cells from five healthy individuals. Almost all CXCR1<sup>+</sup> cells were perforin-positive cells, although perforin-positive cells included both CXCR1<sup>+</sup> and CXCR1<sup>-</sup> cells (Fig. 3A). To analyze semiquantitatively the co-expression of CXCR1 and perforin in CD8<sup>+</sup> T cells, we divided the perforin-positive population into 4–8 fractions according to the level of perforin expression. The frequency of CXCR1<sup>+</sup> cells (Fig. 3B) and expression level of CXCR1 (data not shown) were positively related to perforin expression, demonstrating that CXCR1 expression is strongly associated with perforin expression in CD8<sup>+</sup> T cells. These findings support the idea that CXCR1 is a marker for cytolytic effector CD8<sup>+</sup> T cells.

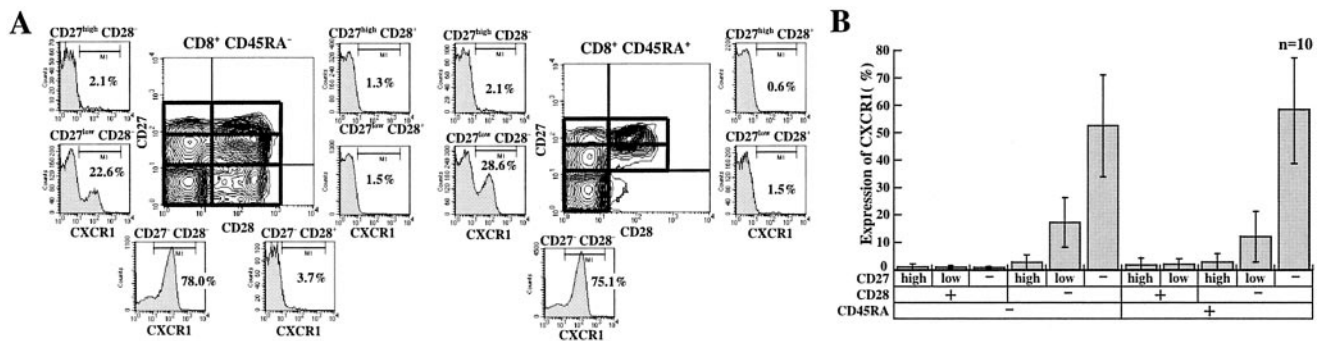
Because CCR5 is expressed on memory and effector CD8<sup>+</sup> T cells (16, 17), it may be assumed that CXCR1<sup>+</sup>CCR5<sup>+</sup> cells would exist among CD8<sup>+</sup> T cells. In fact, CXCR1<sup>+</sup>CCR5<sup>+</sup> cells were found mostly in CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> subsets (data not shown). These CXCR1<sup>+</sup>CCR5<sup>+</sup> cells expressed lower levels of CXCR1 and CCR5 than the CXCR1<sup>+</sup>CCR5<sup>-</sup> cells or CXCR1<sup>-</sup>CCR5<sup>+</sup> cells, suggesting that CXCR1<sup>+</sup>CCR5<sup>+</sup> cells are an intermediate type between the CXCR1<sup>+</sup>CCR5<sup>-</sup> cells and CXCR1<sup>-</sup>CCR5<sup>+</sup> cells in these effector subsets.

HCMV-specific CD8<sup>+</sup> T cells have the CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> effector phenotype or CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>+/-</sup> memory/effector phenotype and the ability to kill target cells (4), whereas EBV-specific CD8<sup>+</sup> T cells have the CD27<sup>+</sup>CD28<sup>+</sup>CD45RA<sup>-</sup> memory phenotype and fail to kill target cells (6), implying that HCMV-specific CD8<sup>+</sup> T cells would express CXCR1 and EBV-specific ones would not. Therefore, we investigated the expression of CXCR1 on HCMV-specific and EBV-specific CD8<sup>+</sup> T cells. PBMCs from individuals U14 and U17 were stained with the HLA-A\*0201/

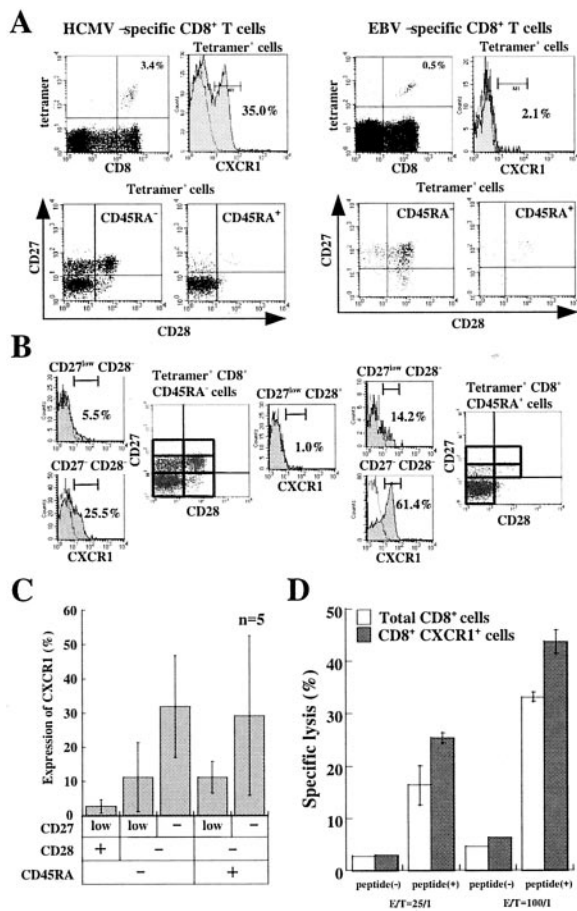


**FIGURE 3.** Coexpression of CXCR1 and perforin in CD8<sup>+</sup> T cells. PBMC from five individuals were stained with anti-CD8, anti-perforin, and anti-CXCR1 or mouse IgG1 mAb. The CD8<sup>+</sup> subset was gated and then analyzed for CXCR1 and perforin expression. N, Mean fluorescence intensity (MFI) between perforin-positive and perforin-negative populations. The perforin-positive population was further divided according to the difference in MFI level as follows: fraction 1, MFI of N × 2<sup>0</sup> to N × 2<sup>1</sup>; fraction 2, MFI of N × 2<sup>1</sup> to N × 2<sup>2</sup>; fraction 3, MFI of N × 2<sup>2</sup> to N × 2<sup>3</sup>; fraction 4, MFI of N × 2<sup>3</sup> to N × 2<sup>4</sup>; fraction 5, MFI of N × 2<sup>4</sup> to N × 2<sup>5</sup>; fraction 6, MFI of N × 2<sup>5</sup> to N × 2<sup>6</sup>; fraction 7, MFI of N × 2<sup>6</sup> to N × 2<sup>7</sup>; and fraction 8, MFI of N × 2<sup>7</sup> to N × 2<sup>8</sup>. A, Coexpression of CXCR1 and perforin in CD8<sup>+</sup> T cells from individuals U23 and U14. B, Percentage of CXCR1<sup>+</sup> cells in the perforin-negative population and in each fraction of the perforin-positive population from five individuals.

HCMV and the HLA-A\*1101/EBV tetramers, respectively, together with anti-CD8 and anti-CXCR1 mAb. HCMV-specific CD8<sup>+</sup> T cells expressed CXCR1, whereas very few EBV-specific CD8<sup>+</sup> T cells expressed this receptor (Fig. 4A). Because HCMV-specific and EBV-specific CD8<sup>+</sup> T cells expressed effector and memory phenotypes, respectively (Fig. 4A), the results indicate that viral epitope-specific CD8<sup>+</sup> T cells



**FIGURE 2.** Surface expression of CXCR1 on CD8<sup>+</sup> T cells with effector and memory/effector phenotypes. A, Frequency of CXCR1<sup>+</sup> cells in each CD27CD28CD45RA subset of CD8<sup>+</sup> T cells. CD8<sup>+</sup> T cells were isolated from a given individual (U25) and then stained with anti-CD27, anti-CD28, anti-CD45RA, and anti-CXCR1 mAbs. Each CD27CD28CD45RA subset was gated and then analyzed for CXCR1 expression. The percentage of CXCR1<sup>+</sup> cells in each subset is shown in each plot. B, Frequency of CXCR1<sup>+</sup> cells in each CD27CD28CD45RA subset of CD8<sup>+</sup> T cells from 10 individuals. The mean percentage and SD of CXCR1<sup>+</sup> cells in each subset are indicated.



**FIGURE 4.** Surface expression of CXCR1 on HCMV-specific and EBV-specific CD8<sup>+</sup> T cells. *A*, Surface expression of CXCR1 on HCMV-specific and EBV-specific CD8<sup>+</sup> T cells. PBMCs from HLA-A\*0201<sup>+</sup> individual (U14) and HLA-A\*1101<sup>+</sup> individual (U17) were stained with the HLA-A\*0201 tetramer and HLA-A\*1101 tetramer, respectively, as well as with anti-CD8 and anti-CXCR1 mAbs. CD8<sup>+</sup>tetramer<sup>+</sup> cells were gated and then analyzed for the expression of CXCR1. CD8<sup>+</sup> T cells from the same individual were also stained with anti-CD27, anti-CD28, and anti-CD45RA mAbs as well as with the HLA-A\*0201 tetramer or the HLA-A\*1101 tetramer. The expression of CD27, CD28, and CD45RA on tetramer<sup>+</sup> cells is shown. *B*, Surface expression of CXCR1 on different CD27/CD28/CD45RA subsets of HCMV-specific CD8<sup>+</sup> T cells. CD8<sup>+</sup> T cells were isolated from individual U14 and then stained with anti-CD27, anti-CD28, anti-CD45RA, and anti-CXCR1 mAbs, as well as with the HLA-A\*0201 tetramer. Each CD27/CD28/CD45RA subset of tetramer<sup>+</sup> cells was gated and then analyzed for CXCR1 expression. The percentage of CXCR1<sup>+</sup> cells in each subset is indicated on each plot. *C*, Frequency of CXCR1<sup>+</sup> cells in different CD27/CD28/CD45RA subsets of HCMV-specific CD8<sup>+</sup> T cells from 5 HLA-A\*0201/-A\*0206<sup>+</sup> individuals. The mean percentage and SD of CXCR1<sup>+</sup> cells in each subset are shown. *D*, Enriching HCMV-specific CTL by sorting CXCR1<sup>+</sup>CD8<sup>+</sup> T cells. Cytotoxic activity of sorted CXCR1<sup>+</sup>CD8<sup>+</sup> T cells (96.6% purity) and total CD8<sup>+</sup> T cells for C1R-A\*0201 cells prepulsed with HCMV-1 pp65<sub>495-503</sub> peptide were measured at E:T ratios of 25:1 and 100:1.

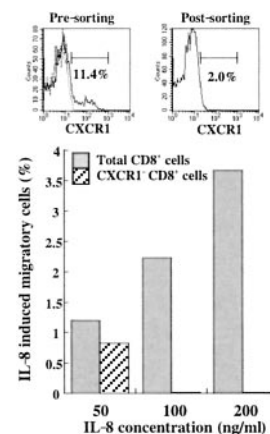
with the effector phenotype expressed CXCR1. Indeed, six-color flow cytometric analysis showed that ~30% of HCMV-specific CD8<sup>+</sup> T cells with the CD27<sup>-</sup>CD28<sup>-</sup>CD45RA<sup>+/+</sup> phenotype expressed CXCR1, but that only 10% of those with the CD27<sup>low</sup>CD28<sup>-</sup>CD45RA<sup>+/+</sup> phenotypes expressed it (Fig. 4, *B* and *C*). The ability of sorted CXCR1<sup>+</sup>CD8<sup>+</sup> T cells to kill HCMV epitope peptide-pulsed cells was ~2-fold higher than that of total CD8<sup>+</sup> T cells (Fig. 4*D*), suggesting that a

considerable number of HCMV-specific CD8<sup>+</sup> T cells with cytotoxic activity expressed CXCR1.

To determine whether chemotaxis of CXCR1<sup>+</sup>CD8<sup>+</sup> T cells is induced by IL-8, we examined the *in vitro* chemotactic activity of CD8<sup>+</sup> T cells purified from a healthy individual. The chemotactic activity of the cells represents as IL-8 induced migratory cells (percentage of migrated cells in absence of IL-8 was subtracted from that in presence of IL-8). Migration of purified CD8<sup>+</sup> T cells was dose-dependently induced by IL-8 with 11.4% of these cells being CXCR1<sup>+</sup>. In contrast, chemotaxis of CXCR1<sup>-</sup>CD8<sup>+</sup> T cells, which were sorted by a cell sorter, was not induced by IL-8 (Fig. 5). Taken together, these results indicate that chemotaxis of CXCR1<sup>+</sup>CD8<sup>+</sup> T cells is induced by IL-8. These results were confirmed in an experiment using a different individual (data not shown). Thus, CXCR1 on CD8<sup>+</sup> T cells has a definite biological function.

There are some differences in the ligand specificity between the two IL-8 receptors. CXCR1 binds IL-8 and granulocyte chemotactic protein (GCP)-2 with high affinity, and growth-related oncogene and neutrophil-activating peptide-2 with low affinity, whereas CXCR2 binds IL-8, GCP-2, growth-related oncogene, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 with high affinity (16). Thus, CXCR1 is the more specific receptor for IL-8. These findings suggest that CD8<sup>+</sup> T cells can respond only to IL-8 and GCP-2 *in vivo*, implying that effector CD8<sup>+</sup> T cells may be specifically attracted to inflammatory sites where IL-8 is produced.

Recent studies in mice showed that leukotriene B<sub>4</sub>, which is produced from mast cells, is involved in the homing of effector CD4<sup>+</sup> and CD8<sup>+</sup> T cells, which express BLT1, a receptor for leukotriene B<sub>4</sub> (17–19), thus suggesting a crucial role for the leukotriene B<sub>4</sub>-BLT1 pathway in allergic inflammation. Our present data also imply a role for the IL-8-CXCR1 pathway in the homing of effector CD8<sup>+</sup> T cells in various kinds of inflammation. Further studies on effector CD8<sup>+</sup> T cell homing via IL-8-CXCR1 pathway may be expected to clarify in the role of this pathway in various infectious diseases.



**FIGURE 5.** Induction of CXCR1<sup>+</sup>CD8<sup>+</sup> T cell migration by IL-8. Migration of total CD8<sup>+</sup> T cells including 11.4% CXCR1<sup>+</sup> cells (presorting CD8<sup>+</sup> T cells) and of sorted CXCR1<sup>-</sup>CD8<sup>+</sup> T cells (after sorting CD8<sup>+</sup> T cells) were induced by different concentrations of IL-8. IL-8 induced migratory cells were calculated as follows: percentage of migrated cells in presence of IL-8 minus that in absence of IL-8.

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