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Functional Expression of the Chemokine Receptor CCR5 on Virus Epitope-Specific Memory and Effector CD8+ T Cells

Katsuhiko Fukada,*† Yuji Sobao,* Hiroko Tomiyama,* Shinichi Oka,‡ and Masafumi Takiguchi*‡

Because the chemokine receptor CCR5 is expressed on Th1 CD4+ cells, it is important to investigate the expression and function of this receptor on other T cells involved in Th1 immune responses, such as Ag-specific CD8+ T cells, which to date have been only partially characterized. Therefore, we analyzed the expression and function of CCR5 on virus-specific CD8+ T cells identified by HLA class I tetramers. Multicolor flow cytometry analysis demonstrated that CCR5 is expressed on memory (CD28+CD45RA−) and effector (CD28−CD45RA− and CD28−CD45RA+) CD8+ T cells but not on naive (CD28+CD45RA+) CD8+ T cells. CCR5 expression was much lower on two effector CD8+ T cells than on memory CD8+ T cells. Analysis of CCR7 and CCR5 expression on the different types of CD8+ T cells showed that memory CD8+ T cells have three phenotypic subsets, CCR5+CCR7−, CCR5−CCR7−, and CCR5+CCR7+, while naive and effector CD8+ T cells have CCR5−CCR7− and CCR5−CCR7+ phenotypes, respectively. These results suggest the following sequence for differentiation of memory CD8+ T cells: CCR5+CCR7−→CCR5+CCR7−→CCR5+CCR7+→CCR5−CCR7+→CCR5−CCR7−. CCR5+CD8+ T cells effectively migrated in response to RANTES, suggesting that CCR5 plays a critical role in the migration of Ag-specific effector and differentiated memory CD8+ T cells to inflammatory tissues and secondary lymphoid tissues. This is in contrast to CCR7, which functions as a homing receptor in migration of naive and memory CD8+ T cells to secondary lymphoid tissues. The Journal of Immunology, 2002, 168: 2225–2232.

Chemokines play well-defined roles in attracting monocytes and immature dendritic cells to sites of inflammation and in directing maturing APCs to lymphatic vessels as part of the initiation of immune responses (1). The expression and function of chemokine receptors on T cells have mostly been partially characterized. Therefore, we analyzed the expression and function of CCR5 on virus-specific CD8+ T cells identified by HLA class I tetramers. Multicolor flow cytometry analysis demonstrated that CCR5 is expressed on memory (CD28+CD45RA−) and effector (CD28−CD45RA− and CD28−CD45RA+) CD8+ T cells but not on naive (CD28+CD45RA+) CD8+ T cells. CCR5 expression was much lower on two effector CD8+ T cells than on memory CD8+ T cells. Analysis of CCR7 and CCR5 expression on the different types of CD8+ T cells showed that memory CD8+ T cells have three phenotypic subsets, CCR5+CCR7−, CCR5−CCR7−, and CCR5+CCR7+, while naive and effector CD8+ T cells have CCR5−CCR7− and CCR5−CCR7+ phenotypes, respectively. These results suggest the following sequence for differentiation of memory CD8+ T cells: CCR5+CCR7−→CCR5+CCR7−→CCR5+CCR7+→CCR5−CCR7+→CCR5−CCR7−. CCR5+CD8+ T cells effectively migrated in response to RANTES, suggesting that CCR5 plays a critical role in the migration of Ag-specific effector and differentiated memory CD8+ T cells to inflammatory tissues and secondary lymphoid tissues. This is in contrast to CCR7, which functions as a homing receptor in migration of naive and memory CD8+ T cells to secondary lymphoid tissues.

Materials and Methods

Abs and reagents

Anti-human CCR5 mAb (2H4), FITC-conjugated anti-human CCR5 mAb (2D7), PE- and allophycocyanin-conjugated anti-human CD28 mAb, CyChrome-conjugated anti-human CD45RA mAb, PerCP-conjugated anti-human CD8 mAb, and FITC- and biotin-conjugated rat anti-mouse IgM mAb were purchased from BD PharMingen (San Diego, CA). Allophycocyanin-conjugated anti-human CD8 mAb was purchased from Beckman Coulter (Fullerton, CA). PE-conjugated extravidin was purchased from Recombinant human RANTES/CCL5 and MIP-1β were purchased from R&D Systems (Minneapolis, MN).
Blood samples

PBMCs from 13 individuals with chronic HIV-1 infection were analyzed in this study. Blood samples were collected with oral informed consent from HLA-A11*, HLA-A24*, and/or HLA-B35* HIV-1-seropositive individuals at the International Medical Center of Japan (Tokyo, Japan). All these individuals belonged to the clinical stage of asymptomatic carrier or AIDS-related complex. PBMCs from 11 HIV-1-seropositive healthy volunteers were also analyzed in this study. Two HLA-A*0201 samples were used for analysis of human CMV (HCMV)-specific CD8+ T cells. Blood samples were also collected with oral informed consent.

Synthetic peptides

Sixteen CTL epitope peptides were synthesized using an automated multiple-peptide synthesizer (Shimadzu Model PSSM-5; Shimadzu, Kyoto, Japan). These peptides were as follows: an HLA-A*0201-restricted HCMV epitope (HCMV-pp65 495–503 NLVPVMATY) (17), an HLA-B*501-restricted hepatitis C virus (HCV) epitope (HCV-NS3 1359–1367 HPNIE EVAL) (18), two HLA-A*1101-restricted HIV epitopes (HIV-Pol 314–321 AlIQFSSMTK, and -Nef 84–92 AVDLVSHFLK) (19, 20), five HLA-A24*0202-restricted HIV epitopes (HIV-Nef 138–147 RYPLIFTGWCF, -Nef 138–147 FRPLTGWCIF, -env 584–592 KYRLDRQQL, -env 679–687 WYKJKKK, and -Gag 28–36 KYYVKHHWV) (21, 22), and two HLA-B*3501-restricted HIV epitopes (HIV-Pol 275–282 V2PLDKFRKY, -Nef 75–85 RPQVPILRPMY, -Pol 448–456 IPTLIEEAL, -Pol 587–595 EPIVGAEFT, and -env 77–85 DPNOQPEVVL) (23) and two HLA-A*1101-restricted EBV epitopes (EBV-3B 416–424 IVTDFSVIK, and EBV-3B 399–408 AVFDKRSKDAK) (24). All peptides were verified to be >90% pure by mass spectrometry and HPLC.

Production of HLA class I peptide tetramers

HLA-A*0201, HLA-A*1101, HLA-A*2402, and HLA-B*3501 peptide tetrameric complexes were prepared as previously described (25, 26). Briefly, recombinant human β2-microglobulin and recombinant HLA class I derivatives (COOH termini of HLA class I molecules with truncated transmembrane and cytoplasmic domains, and with a sequence containing the BirA enzymatic biotinylation site) were purified from Escherichia coli cells and used in the relevant expression plasmid. Monomeric complexes were generated by in vitro refolding of human β2-microglobulin, the appropriate HLA class I derivative, and an HIV-1-specific, EBV-specific, or HCMV-specific epitope peptide. The resultant 45-kDa complexes were separated by gel filtration using a Superdex G75 column (Amersham Pharmacia Biotech, Uppsala, Sweden) and then biotinylated enzymatically with BirA enzyme (AviTag, Denver, CO). The biotin-binding complexes were separated by gel filtration using a Superdex G75 column followed by anion exchange using a Mono Q column (Amersham Pharmacia Biotech). HLA class I/peptide tetramers were generated by mixing the monomer complexes with PE-conjugated extravidin (Sigma Chemical) or allophycocyanin-conjugated streptavidin (BD Pharmingen) at a molar ratio of 4:1.

CTL clones


Flow cytometry analyses

Cryopreserved PBMCs from HIV-1-seropositive individuals and healthy subjects were stained with mAbs and/or tetramers in various combinations. PBMCs from HIV-1-seronegative and -seropositive individuals were stained with FITC-conjugated anti-CCR5 mAb and PerCP-conjugated anti-CD8 mAb, with those from HIV-1-seropositive individuals further stained with allophycocyanin- or PE-conjugated tetramer(s). Incubation with tetramer(s) and subsequent washing were performed at 37°C to avoid non-specific binding to CD8+ T cells, as previously described (27). For four-color flow cytometry, PBMCs isolated from healthy individuals were stained with PE-conjugated anti-CD28, CyChrome-conjugated anti-CD45RA, and allophycocyanin-conjugated anti-CD8. Similarly, after CD8+ T cells were purified from PBMCs of HIV-1-seronegative and -seropositive individuals using the MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany), these cells were stained with FITC-conjugated anti-C3R5 mAb, PE-conjugated anti-CD28 mAb, CyChrome-conjugated anti-CD45RA mAb, and allophycocyanin-conjugated tetramer(s) for HCMV and HIV-1, respectively. CTL clones were stained with FITC-conjugated anti-C3R5 mAb and PerCP-conjugated anti-CD8 mAb. To investigate CCR7 expression on CD8+ T cells and Ag-specific CD8+ T cells, purified CD8+ T cells from HCMV-seropositive healthy individuals were stained with anti-CCR7 mAb followed by FITC-conjugated rat anti-mouse IgM mAb, PE-conjugated anti-CD28 mAb, CyChrome-conjugated anti-CD45RA, and allophycocyanin-conjugated tetramer(s) for HCMV. To investigate coexpression of CCR7 and CCR5 on CD8+ T cells, purified CD8+ T cells from HCMV-seropositive healthy individuals were stained with anti-CCR7 mAb followed by biotin-conjugated rat anti-mouse IgM mAb and then PE-conjugated extravidin, FITC-conjugated anti-CCR5 mAb, CyChrome-conjugated anti-CD45RA mAb, and allophycocyanin-conjugated anti-CD28 mAb. Cells were washed three times with PBS supplemented with 10% FCS and then resuspended with PBS containing 2% paraformaldehyde. Samples were analyzed by FACS Calibur with CellQuest software (BD Biosciences, San Jose, CA).

CTL assay

CTL activity was measured by a standard 3Cr release assay as follows. The HLA-A,B defective cell line C1R as well as the C1R transfectants C1R-A*1101 and C1R-A*0201 (5 × 10^5 cells) were incubated for 60 min with 3.7 M Bq Na^2^CrO_4 and then washed three times with RPMI 1640 medium containing 10% newborn calf serum. Labeled target cells (2 × 10^5/well) were added into U-bottom 96-well microtiter plates with 10 μM HCMV (HCMV-pp65 495–503) or two EBV (EBV-3B 416–424 and EBV 3B 399–408) peptides. After incubation for 1 h, CD8+ T cells purified from PBMC of each donor Maximum 3Cr release was determined by measuring the cpm in supernatants from wells containing target cells in the presence of 2.5% Triton X-100 (cpm max). Specific lysis = ([cpm exp – cpm spn]/cpm max – cpm spn) × 100, where cpm exp is the cpm in supernatants of wells containing both target and effector cells.

Lymphocyte chemotaxis assay

The lymphocyte chemotaxis assay was performed using 96-well microchemotaxis chambers (NeuroProbe, Gaithersburg, MD) as previously described (28). The MACS magnetic cell separation system was used to isolate CD8+ T cells from fresh PBMCs taken from healthy individuals. More than 98% of the purified cells were CD8+ T cells. The purified CD8+ T cells (5 × 10^5 cells/well) were placed over the filter (5-μm pore diameter) in the upper wells of the chamber. Recombinant human RANTES/CCL5 and an anti-human CCR5 mAb were diluted with RPMI 1640 and then applied to either the upper or lower wells of the chamber. After incubation at 37°C for 2 h, cells remaining above the filter (i.e., cells that did not migrate) were removed by washing with PBS containing 2 mM EDTA and washing. Cells that had migrated to the lower well of the chamber and below the filter were collected by centrifugation. These cells were counted using a hemocytometer and then stained with PE-conjugated anti-CD8 mAb and CyChrome-conjugated anti-CD45RA mAb for flow cytometry analysis. The percentage of migrated cells with each CD25/CD45RA phenotype was determined as follows: the total number of cells with each phenotype added to the upper well was calculated from total number of cells added to the upper well and the percentage of each phenotype determined by FACS analysis. The migrated cell number of each phenotype was calculated from both the total number of cells migrated to the lower well and the percentage of each phenotype. The phenotype was determined as (migrated cell number with each phenotype/total cell number added to the upper wells with each phenotype) × 100.

Results

Expression of CCR5 on HIV-1-specific CD8+ T cells

We used flow cytometry to investigate the surface expression of CCR5 on total CD8+ T cells in PBMCs isolated from 11 healthy individuals. A significant number of total CD8+ T cells expressed CCR5 (Fig. 1A). Expression varied among individuals, with the per-
HIV-1-seropositive individuals (CD8+ T cells from HIV-1-seronegative healthy and HIV-1-seropositive individuals) were tetramer positive. Tetramer CD8+ T cells were detected by three HLA-B*3501 tetramers in PBMCs from KI-003. These tetramer CD8+ T cells expressed CCR5 (Fig. 1, B, right panel). Analysis of PBMCs from 11 HIV-1-infected individuals showed that the majority of HIV-1-specific CD8+ T cells expressed CCR5 (mean, 63.7 ± 17.0%; Fig. 1C). CCR5 expression was similarly observed on HCMV-specific CD8+ T cells (data not shown). CCR5 was also expressed on HIV-1-specific and HCV-specific CD8+ CTL clones (Fig. 2), further confirming that Ag-specific CD8+ T cells express CCR5.

CCR5 expression changes during CD8+ T cell differentiation

A previous study showed that CD27–CD45RA+CD8+ T cells have neither perforin nor cytolytic activity while CD27+CD45RA–CD8+, CD27–CD45RA–CD8+, and CD27–CD45RA+CD8+ T cells have...
CD28CD45RA subsets. HCMV-specific (25). Therefore, we investigated the cytolytic activity of these cells have a low, medium, and high level of perforin, respectively.

We also investigated CCR5 expression in the four CD8+ T cell populations using Ag-specific CD8+ T cells isolated from HIV-1-seropositive individuals and HCMV-seropositive individuals. CD8+ T cells were isolated from PBMCs using anti-CD8 mAb-coated immunobeads, costained with tetramers, anti-CD28 mAb, anti-CD45RA mAb, and anti-CCR5 mAb, and analyzed by flow cytometry. These analyses showed that in HIV-1- and HCMV-specific T cells, CD28+CD45RA−CD8+ T cells express high levels of CCR5 while CD28+CD45RA−CD8+ and CD28−CD45RA−CD8+ T cells express lower levels of CCR5 (Fig. 4). Thus, in both HIV-1-seropositive and HCMV-seropositive individuals, CCR5 expression increases during differentiation of naive CD8+ T cells to memory CD8+ T cells and then decreases during differentiation to effector CD8+ T cells.

**Coexpression of CCR5 and CCR7 on memory CD8+ T cells**

Recent studies demonstrated that CCR7 is expressed on naive (CD27−CD45RA+) and memory (CD27+CD45RA−) CD8+ T cells (30). We analyzed CCR7 expression on the different CD8+ T cell populations from three healthy individuals. Results from a representative individual are shown in Fig. 5A. CD28+CD45RA−CD8+ T cells and ~50% of CD28+CD45RA−CD8+ T cells express CCR7 while CD28+CD45RA−CD8+ and CD28−CD45RA−CD8+ T cells did not express CCR7. Thus, we confirmed that naive and memory CD8+ T cells express CCR7. To clarify whether Ag-specific memory CD8+ T cells express CCR7, we analyzed CCR7 expression on HCMV-specific CD8+ T cells isolated from two HCMV-seropositive individuals. These results showed that in HCMV-specific CD8+ T cells, CD28−CD45RA+CD8+ and CD28−CD45RA−CD8+ T cells did not express CCR7 while a small population of CD28+CD45RA−CD8+ T cells did express CCR7 (Fig. 5B), suggesting that HCMV-specific memory CD8+ T cells may be well differentiated.

A large percentage of memory T cells expressed CCR5 while only ~50% expressed CCR7, suggesting that the memory CD8+ T cell population has at least two different subsets. Therefore, we further analyzed expression of CCR5 and CCR7 on CD28+CD45RA−CD8+ T cells. Results from three individuals demonstrated the existence of three subsets of CD28+CD45RA− memory CD8+ T cells; CCR5+CCR7−, CCR5−CCR7−, and CCR5+CCR7− (Fig. 6). The expression level of CCR5 in the CCR5+CCR7− subset was higher than that in the CCR5−CCR7− subset, while the expression level of CCR7 in the CCR5+CCR7− subset was higher than that in the CCR5−CCR7− subset. CCR5 was expressed on CD28+CD45RA−CD8+ T cells but not on CD28+CD45RA+CD8+ T cells, while CCR7 was expressed on CD28+CD45RA−CD8+ T cells but not on CD28+CD45RA+CD8+ T cells. These findings strongly suggest the following differential lineage in CD28+CD45RA− memory CD8+ T cells: CCR5+CCR7−→CCR5−CCR7−→CCR5−CCR7−.

Table 1. CCR5 expression on CD8+ T cells with different CD28/CD45RA phenotypes in PBMCs isolated from eight healthy individuals

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Percentage of CCR5+ T cells</th>
<th>MFIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28+CD45RA−</td>
<td>3.6 ± 2.6</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>CD28−CD45RA−</td>
<td>82.6 ± 7.5</td>
<td>28.6 ± 9.0</td>
</tr>
<tr>
<td>CD28+CD45RA+</td>
<td>53.2 ± 20.8</td>
<td>9.5 ± 3.7</td>
</tr>
<tr>
<td>CD28−CD45RA+</td>
<td>42.4 ± 22.1</td>
<td>7.7 ± 4.4</td>
</tr>
</tbody>
</table>

a Data (mean ± SD) were calculated from the percentage of CCR5+ T cells in total CD8+ T cells in PBMCs isolated from eight HIV-1-seronegative, healthy individuals.

MFI (mean ± SD) for CCR5 expression in CD8+ T cells with each phenotype from eight HIV-1-seronegative, healthy individuals.
Effective migration of memory and effector CD8⁺ T cells

Migration of virus-specific memory and effector CD8⁺ T cells to regions of viral replication seems to be one of the most important events in virus eradication. As CCR5 is a receptor for the β-chemokines RANTES, MIP-1α, and MIP-1β, CCR5⁺ CD8⁺ T cells could be expected to actively migrate to inflammatory regions where these chemokines are produced. To clarify the function of CCR5 in CD8⁺ T cells, we investigated RANTES-mediated migration of CCR5⁺ CD8⁺ T cells. CD8⁺ T cells were isolated from PBMCs of a healthy individual and then added to the upper wells of a 96-well microchemotaxis chamber. The CD28/CD45RA phenotype of cells that migrated to the bottom well of the chambers was determined by staining with anti-CD28 mAb and anti-CD45RA mAb followed by flow cytometry. An effect of RANTES on CD8⁺ T cell migration was observed for CD28⁺CD45RA⁻, CD28⁻CD45RA⁻, and CD28⁻CD45RA⁺ populations in a dose-dependent fashion (Fig. 7A). This effect was stronger on the CD28⁻CD45RA⁺ population than on the other two populations. These results were confirmed by three independent experiments. The effect of RANTES on these three populations was abrogated by an anti-CCR5 mAb (Fig. 7B). These three populations did not express CCR1, another receptor for RANTES (data not shown). MIP-1β had a similar effect on these three populations (data not shown). MIP-1β also had a weak effect on the CD28⁻CD45RA⁺ population (data not shown), suggesting that this population may express another receptor for MIP-1β. These results together suggest that memory and effector CD8⁺ T cells can actively migrate in response to the β-chemokines RANTES and MIP-1β.

Discussion

A previous study, which investigated the functions (cytolytic activity, cytokine production, and perforin expression) of CD8⁺ T cell CD27⁺CD45RA⁻ subsets, suggested that CD27⁺CD45RA⁻CD8⁺ T cells are naive cells, while CD27⁺CD45RA⁺CD8⁺, CD27⁻CD45RA⁻CD8⁺, and CD27⁻CD45RA⁺CD8⁺ T cells are memory, memory/effector, and effector cells, respectively (29). This study as well as our recent study (25), which investigated perforin expression and cytolytic activity of CD28CD45RA subsets, showed the same correlation between CD28CD45RA phenotypes and functional classifications (naive, memory, memory/effector, and effector cells). In addition, we have found that CD28⁺CD45RA⁻CD8⁺, CD28⁻CD45RA⁻CD8⁺, and CD28⁻CD45RA⁺CD8⁺ T cells have the capacity to produce cytokines (our unpublished observation). Thus, because CD28 and CD45RA are useful molecules for discriminating naive, memory, memory/effector, and effector CD8⁺ T cells, we used these markers to investigate the expression of CCR5 and CCR7 on naive, memory, memory/effector, and effector CD8⁺ T cells.

Expression of CCR5 on CD8⁺ T cells is thought to be important in Th1 immune responses associated with β-chemokines. Though previous studies showed that some CD8⁺ T cell populations express CCR5 (11–14), in this study we demonstrated that CCR5 expression is restricted to CD28⁺CD45RA⁻CD8⁺, CD28⁻CD45RA⁻CD8⁺, and CD28⁻CD45RA⁺CD8⁺ T cell populations, i.e., memory and effector CD8⁺ T cells but not naive CD8⁺ T cells express CCR5. CD8⁺ T cells have been shown to express the chemokine receptor CCR7 (15). A previous study suggested the following differentiation lineage for Ag-specific CD8⁺ T cells: CCR7⁺CD45RA⁻→CCR7⁺CD45RA⁻→CCR7⁺CD45RA⁻→CCR7⁺CD45RA⁻ (16). Furthermore, a recent study showed that CCR7 is expressed on CD27⁺CD45RA⁻CD8⁺ and CD27⁻CD45RA⁻CD8⁺ but not on CD27⁻CD45RA⁻CD8⁺ T cells (31), suggesting that CCR7 is expressed only on naive and memory CD8⁺ T cells. The present study

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** CCR5 expression on HIV-1-specific and HCMV-specific CD8⁺ T cells with each CD28/CD45RA phenotype. CD8⁺ T cells were purified from three HIV-1-seropositive individuals (A, KI-003; B, KI-005; and C, KI-011) and an HCMV-seropositive healthy individual (D). The purified cells (>98% pure) were costained with tetramers (HLA-A*0201, HLA-A*1101, HLA-A*2402, and/or HLA-B*3501 tetramers), anti-CD28 mAb, anti-CD45RA mAb, and anti-CCR5 mAb. Tetramer⁺ CD8⁺ T cells were analyzed for CD28/CD45RA expression and then CCR5 expression in three CD28 and CD45RA phenotypes (CD28⁺CD45RA⁻, CD28⁻CD45RA-, and CD28⁻CD45RA⁺) was measured. The numbers show the MFI for cells of each CD28/CD45RA phenotype stained with (bold text) or without (normal text) anti-CCR5 mAb.
also demonstrated that all CD28\(^{+}\)/CD45RA\(^{+}\)/CD8\(^{+}\) T cells and approximately half of CD28\(^{+}\)/CD45RA\(^{-}\)/CD8\(^{+}\) T cells express CCR7 while CD28\(^{-}\)/CD45RA\(^{-}\)/CD8\(^{+}\) T cells and CD28\(^{-}\)/CD45RA\(^{+}\)/CD8\(^{+}\) T cells do not. Analysis of CCR5 and CCR7 coexpression demonstrated three CD28\(^{+}\)/CD45RA\(^{-}\) memory CD8\(^{+}\) T cell subsets, CCR5\(^{+}\)CCR7\(^{+}\), CCR5\(^{-}\)CCR7\(^{-}\), and CCR5\(^{-}\)CCR7\(^{+}\). Naive and effector CD8\(^{+}\) T cells are CCR5\(^{+}\)CCR7\(^{-}\) and CCR5\(^{-}\)CCR7\(^{-}\), respectively. These results indicate the following differential lineage of

FIGURE 5. Expression of CCR7 on total CD8\(^{+}\) T cells and virus-specific CD8\(^{+}\) T cells with different CD28/CD45RA phenotypes. A, PBMCs purified from healthy individuals (U-1 and U-2) were costained with anti-CD8, anti-CD28, anti-CD45RA, and anti-CCR7 mAb and analyzed by flow cytometry. CD8\(^{+}\) T cells were first analyzed for CD28 and CD45RA expression, and then CCR7 expression on each CD28/CD45RA phenotype (CD28\(^{+}\)/CD45RA\(^{+}\), CD28\(^{+}\)/CD45RA\(^{-}\), CD28\(^{-}\)/CD45RA\(^{+}\), and CD28\(^{-}\)/CD45RA\(^{-}\) cells) was measured. B, CD8\(^{+}\) T cells were purified from HCMV-seropositive HLA-A\(^{*}\)0201 healthy individuals (U-1 and U-2). The purified cells (>98% pure) were costained with HCMV tetramer, anti-CD28 mAb, anti-CD45RA mAb, and anti-CCR7 mAb. Tetramer\(^{+}\)CD8\(^{+}\) T cells were analyzed for CD28 and CD45RA expression and then CCR7 expression in three CD28/CD45RA phenotypes (CD28\(^{+}\)/CD45RA\(^{+}\), CD28\(^{-}\)/CD45RA\(^{+}\), and CD28\(^{-}\)/CD45RA\(^{-}\) ) was measured.

FIGURE 6. Three memory CD8\(^{+}\) T cell subsets have different CCR5/CCR7 phenotypes. CD8\(^{+}\) T cells were purified from three healthy individuals (U-1, U-2, and U-4). The purified cells (>98% pure) were analyzed for CD28 and CD45RA expression and then CCR5 and CCR7 expressions in four CD28/CD45RA subsets (CD28\(^{+}\)/CD45RA\(^{+}\), CD28\(^{+}\)/CD45RA\(^{-}\), CD28\(^{-}\)/CD45RA\(^{+}\), and CD28\(^{-}\)/CD45RA\(^{-}\)) were measured. The numbers show the percentages of cells in each quadrant. MFI for CCR5 and CCR7 is as follows. Individual U-1: CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset; CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset; CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset; CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset. Individual U-2: CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset; CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset; CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset. Individual U-4: CCR5\(^{+}\)CCR7\(^{+}\) subset, CCR5\(^{+}\)CCR7\(^{-}\) subset, CCR5\(^{-}\)CCR7\(^{+}\) subset, CCR5\(^{-}\)CCR7\(^{-}\) subset.
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