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CHAPTER 6

PROLIFERATION REQUIREMENTS OF CYTOMEGALOVIRUS-SPECIFIC, EFFECTOR-TYPE HUMAN CD8+ T CELLS

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Proliferation Requirements of Cytomegalovirus-Specific, Effector-Type Human CD8⁺ T Cells

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Two prototypic types of virus-specific CD8⁺ T cells can be found in latently infected individuals: CD45R0⁺CD27⁻CCR7⁻ effector-memory, and CD45RA⁺CD27⁻CCR7⁻ effector-type cells. It has recently been implied that CD45RA⁺CD27⁻CCR7⁻ T cells are terminally differentiated effector cells and as such have lost all proliferative capacity. We show in this study, however, that stimulation of CMV-specific CD45RA⁺CD27⁻CCR7⁻ T cells with their cognate peptide in concert with either CD4⁺ help or IL-2, IL-15, or IL-21 in fact induces massive clonal expansion. Concurrently, these stimulated effector T cells change cell surface phenotype from CD45RA to CD45R0 and regain CCR7, while effector functions are maintained. Our data imply that CD45RA⁺CD27⁻CCR7⁻ effector-type T cells contribute to immunity not only by direct execution of effector functions, but also by yielding progeny in situations of viral reinfec­tion or reactivation. The Journal of Immunology, 2002, 169: 5838–5843.

Immuneological memory is the key feature of the adaptive immune system and provides organisms with faster and more efficient means to cope with offensive pathogens on a secondary encounter. Immunological memory has a cellular basis: in response to antigenic challenge, numbers of Ag-specific lymphocytes rise (1), and these expanded cells have an increased affinity for their cognate peptide in concert with either CD4⁺ help or IL-2. Naïve cells activated by Ag and costimulatory signals develop into a greatly expanded pool of effector T cells that eliminates or neutralizes the pathogen. The majority of these effector cells dies, but a fraction persists as memory cells. A distinct theory suggests that effector and memory cells arise from the same precursor cell, but differentiate along separate pathways (3–5).

The discussion on the development of T cell memory has been fueled by recent observations on human virus-specific T cells (6, 7). Essentially, two prevalent sets of primed Ag-specific CD8⁺ T cells can be distinguished during viral latency (8–10). First, there are CD45R0⁺CD27⁻CCR7⁻ T cells that have been designated effector-memory cells. The majority of these T cells express the costimulatory molecule CD28, and these cells do not abundantly express cytolytic mediators such as perforin and granzyme B. Second, other Ag-specific CD8⁺ T cells have a CD45RA⁺CD27⁻CCR7⁻ phenotype, express both perforin and granzyme B, and are able to execute direct ex vivo cytolysis. In healthy individuals, cells belonging to this subset also lack the costimulatory receptor CD28 (11, 12), and in many studies the absence of this marker has been used to identify effector-type cells (13, 14). The reason for the apparent dichotomy in phenotype and function of virus-specific T cells during latency is unclear. Recent data suggest that particular pathogens may preferentially induce the appearance of certain subsets (7). In contrast, it is clear that in healthy individuals phenotypically distinct CMV-specific T cells are found (8).

CD8⁺CD45RA⁺CD27⁻CCR7⁻ effector T cells have been termed terminally differentiated CD8⁺ T cells (6, 15). This implies that these lymphocytes, in analogy to cells in other organ systems such as the skin and the gastrointestinal tract, have lost the potential to self-renew and expand. Several observations supported the notion that effector CD8⁺ T cells have irresponsibly lost proliferative capacity. The nuclear Ag Ki-67, which is expressed in all phases of the cell cycle except the G₀ phase, could not be demonstrated in effector T cells of HIV and EBV patients (6, 12). CD8⁺CD28⁻ T cell populations, and T cell clones did not show anti-CD3 mAb-induced proliferation, and this deficiency could not be overcome by the addition of exogenous IL-2; neither could these cells be costimulated using CD80 or CD58 (13). Furthermore, no evidence for significant cell division was observed in CD8⁺ CD45RA⁺CCR7⁻ cells upon stimulation with anti-CD3 and anti-CD28 mAbs, as measured by CFSE labeling (6). In contrast, we reported that CD45RA⁺CD27⁻ effector-type cells do divide when stimulated with CD2 mAbs in combination with IL-2 and, to a lesser extent, IL-15 (11). Moreover, a recent report suggested that stimulation of a CMV-specific CD8⁺ T cell population largely containing CD45RA⁺CD27⁻CCR7⁻ T cells with specific peptide and IL-2 resulted in proliferation and differentiation of effector-type T cells (14). To evaluate their potential role in virus elimination in situations of viral reactivation or reinfection, we in this study investigated whether CMV-specific effector-type CD8⁺CD45RA⁺CD27⁻CCR7⁻ T cells expand in response to both specific peptide and CD4⁺ T cell-derived helper signals. Our data show that although CD8⁺CD45RA⁺CD27⁻CCR7⁻ CMV-specific T cells have all features of effector cells, they have not lost the ability to expand upon viral challenge.

Materials and Methods

Isolation of Peripheral Blood Mononuclear Cells

Heparinized peripheral blood samples were collected from HLA-A2-positive CMV-seropositive healthy donors and renal transplant recipients from the Academic Medical Center transplantation outpatient clinic. All patients gave written informed consent, and the study was approved by the local committee of the Academic Medical Center.

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medical ethical committee. Diluted blood was layered on Lymphoprep (Nycomed, Pharma, Oslo, Norway) for density gradient centrifugation, and PBMCs were harvested from the interface and washed twice. Cells were frozen and stored in liquid nitrogen until the day of analysis.

Generation of tetrameric complexes

Tetrameric complexes were generated essentially as described previously (16). In brief, purified HLA-A2.1 H chain and β2-microglobulin were synthesized using a prokaryotic expression system (pET; Novagen, Milwaukee, WI). The H chain was modified by deletion of the transmembrane/cytosolic tail and C-terminal addition of a sequence containing the BirA enzymatic biotinylation site. The HLA-A2.1-binding CMVpp65-derived peptide NLVPTMVATV was used for refolding. The refolded product was isolated by fast protein liquid chromatography, and biotinylated by BirA (expressed using the pET expression system and purified using Clontech cobalt beads, Palo Alto, CA) in the presence of biotin (Sigma-Aldrich, St. Louis, MO), ATP (Sigma-Aldrich), and MgCl₂. Streptavidin-allophycocyanin conjugate (Molecular Probes, Europe BV, Leiden, The Netherlands) was added in a 1:4 molar ratio.

Flow cytometric analyses

Thawed PBMCs were resuspended in IMDM (BioWhittaker, Verviers, Belgium), containing 10% FCS and antibiotics (100 U/ml sodium penicillin G (Bocoreas pharma B.V., Leiden, The Netherlands) and 100 μg/ml streptomycin sulfate (Life Technologies, Paisley, Scotland)). Cells were washed in PBS containing 0.01% (w/v) NaN₃ and 0.5% (w/v) BSA (PBA). A total of 250,000 PBMCs were incubated with an appropriate concentration of tetrameric complexes in a small volume for 10 min at 4°C, protected from light. Then fluorescent-labeled conjugated mAbs (concentrations according to manufacturer's instructions) were added and incubated for 30 min at 4°C, protected from light. For analysis of expression of surface markers, the following Abs were used in different combinations: allophycocyanin-conjugated tetramers, CD45RA FITC (BD Biosciences, San Jose, CA), CD45RA PE (Sanquin, Amsterdam, The Netherlands, or Coulter, Miami, FL), CD45RO PE, CD27 PE, CD8 FITC, CD8 PE, CD8 allophycocyanin, and CD8 PerCP (all BD Biosciences).

For staining with the murine anti-humanCCR7 mAb, a multistep staining protocol was performed consisting of incubation with the anti-CCR7 Ab (BD Pharmingen, San Diego, CA) for 30 min at 4°C protected from light, followed by biotinylated rat anti-mouse IgM (BD Pharmingen) for 30 min at 4°C protected from light, incubation with streptavidin-PE (BD Biosciences) for 30 min at 4°C protected from light, incubation with 10% (v/v) normal mouse serum (Sanquin), followed by incubation with tetrameric complexes and incubation with directly conjugated mAbs, as described above.

Cells were washed in PBA and analyzed using a FACSCalibur flow cytometer and CellQuest software (BD Biosciences).

Intracellular granzyme B, perforin, and cytokine staining

Intracellular granzyme B and perforin stainings were performed, as described previously (17). In short, 250,000 PBMCs were stained with fluorescent-labeled conjugated mAbs to CD8 and CMV-tetramer complexes, washed once with PBA, then fixed with 50 μl buffered formaldehyde acetone solution, and subsequently permeabilized by washing with 0.1% saponin, 50 mM N-glycine. Cells were then incubated with anti-granzyme B PE (Sanquin) or anti-perforin PE (Holzel Diagnostika, Köl, Germany) Abs, according to manufacturers' instructions. Flow cytometric analysis was performed thereafter.

For the cytokine staining, cells were first stimulated in a 24-well plate at 1×10⁶ cells/well in 0.5 ml with PMA, ionomycin, and brefeldin A for 4 h at 37°C. Cells were washed, and then the same staining procedure was performed as described above, but performed using anti-IFN-γ or anti-TNF-α Abs either FITC or PE labeled (all from BD Biosciences).

Depletion of CD4+ T cells

PBMCs were incubated with unlabeled anti-CD4 Abs for 30 min at 4°C protected from light. After washing, 4×10⁶ Dynabeads (M-450, goat anti-mouse IgG (Dynal ASA, Oslo, Norway)) were added per 1×10⁶ PBMCs at a final concentration of 1×10⁶ PBMCs/ml. Cells were then incubated for 30 min at 4°C, and subsequent magnetic depletion of CD4+ T cells was performed. Identity of the remaining fraction was checked by flow cytometry: ≥95% of CD4+ cells were CD8−, and monocytes were retained in the resulting cell population.

CFSE labeling

PBMCs were pelleted and resuspended in PBS at a final concentration of 5-10×10⁶ cells/ml. PBMCs were labeled with 2.5 μM (final concentration) of CFSE (Molecular Probes) in PBS for 5 min shaking at 37°C. Cells were washed and subsequently resuspended in IMDM supplemented with 10% human pool serum, antibiotics, and 5.37×10⁻⁶ % (v/v) 2-ME (Merck, West Point, PA) (culture medium).

Culture and stimulation of the cells

CFSE-labeled cells were cultured in culture medium for 5 days in 24-well plates at a concentration of 0.5-1×10⁶ cells/ml. CMVpp65-derived peptide was added at a final concentration of 1.25 μg/ml CMV Ag (inactivated whole virus, 60 μg/ml, BioWhittaker) and purified protein derivative (PPD, 11.8 μg/ml; Statens Serum Institut, Copenhagen, Denmark) were used to stimulate cells. Furthermore, for stimulation, IL-2 (50 U/ml; Biozol Ag, Dreieich, Germany), IL-4 (10 ng/ml; Strathmann Bioche GmbH, Hamburg, Germany), IL-7 (10 ng/ml; Strathmann, IL-10 (100 U/ml; BD Pharmingen), IL-12 (10 ng/ml; Strathmann), IL-15 (3 ng/ml; R&D Systems, Abingdon, U.K.), IL-21 (50 ng/ml; Zymogenetics, Seattle, WA), IFN-γ (100 U/ml), or TNF-α (25 ng/ml) (both R&D Systems) was added.

For blocking experiments, we used anti-IL-2R α-chain (1:250; Sanquin). Flow cytometric analysis was performed before culture and after 5 days.

Cytotoxic assays

Cells were cultured with different stimulatory conditions for 5 days, as described above. The percentage of tetramer-positive cells was measured by flow cytometric analysis. CTL activity was measured in a standard ¹²⁵I release assay using an HLA A2.1 EBV cell line as a target. Briefly, peptide-coated cell line target cells were prepared by labeling cells with 50 μl 30 μg/ml ¹²⁵I (Crim, 500 μCi/ml for 1 h at 37°C, 5% CO₂. After washing, cells were incubated with 0.1 mg/ml peptide or medium for 1 h at 37°C, 5% CO₂. Graded numbers of effector cells were incubated with 3×10⁶ ¹²⁵I-labeled target cells for 4 h at 37°C, 5% CO₂ in different E/T ratios. After incubation, released radioactivity was measured and specific lysis was calculated according to the following formula: percentage of specific release = ([experimental release − spontaneous release] / maximum release − spontaneous release) × 100. Results are presented as specific lysis adjusted for the number of tetramer-binding cells in the population.

Results

CMV-specific effector-type CD8+ T cells expand upon antigen stimulation

CMV-specific CD8+ T cells can be directly visualized using tetrameric HLA-A2.1-NLVPMTAVT complexes (Fig. 1). As previously demonstrated, the CMV tetramer-positive cells from a number of healthy individuals and from most renal transplant recipients predominantly have an effector cell phenotype (8), being CD8+ CD45RA- CD27- CD28- CD27 PE, CD8 FITC, CD8 PE, CD8 allophycocyanin, and CD8 PerCP (all BD Biosciences).

Intracellular granzyme B, perforin, and cytokine staining

Intracellular granzyme B and perforin stainings were performed, as described previously (17). In short, 250,000 PBMCs were stained with fluorescent-labeled conjugated mAbs to CD8 and CMV-tetramer complexes, washed once with PBA, then fixed with 50 μl buffered formaldehyde acetone solution, and subsequently permeabilized by washing with 0.1% saponin, 50 mM N-glycine. Cells were then incubated with anti-granzyme B PE (Sanquin) or anti-perforin PE (Holzel Diagnostika, Köln, Germany) Abs, according to manufacturers' instructions. Flow cytometric analysis was performed thereafter.

For the cytokine staining, cells were first stimulated in a 24-well plate at 1×10⁶ cells/well in 0.5 ml with PMA, ionomycin, and brefeldin A for 4 h at 37°C. Cells were washed, and then the same staining procedure was performed as described above, but performed using anti-IFN-γ or anti-TNF-α Abs either FITC or PE labeled (all from BD Biosciences).

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Abbreviation used in this paper: PPD, purified protein derivative.

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FIGURE 1. CMV-specific CD8+ T cells have an effector cell phenotype and can proliferate. A. Phenotype of unstimulated cells. B. CFSE dilution of cells cultured for 5 days, in medium, with CMV peptide, CMV Ag, or CMV peptide plus CMV Ag, respectively. All plots are gated on CD8+ T cells. C. Phenotype of cells stimulated for 5 days with CMV peptide and CMV Ag. D. Phenotype of cells stimulated for 5 days with CMV peptide and IL-2. A, C, and D. The first dot plots are gated on lymphocytes; the other dot plots are gated on CD8+ T cells. The numbers in the quadrants are the percentages of total tetramer+ cells. Representative flow cytometry is shown of n = 15 for phenotype CMV-specific cells at day 0, n = 9 for CFSE stainings, and n = 6 for phenotype CMV-specific cells after stimulation with CMV peptide and CMV Ag or IL-2.

However, stimulation with CMV peptide plus CMV Ag resulted in strong proliferation of all tetramer-positive cells (Fig. 1B). This is indicated not only by the diminished intensity of the CFSE staining of the cells, but also by the increase of the total amount of CMV-specific cells (mean 38% of CD8+ T cells, range 15–59%, n = 9 independent experiments) compared with the starting population (mean 5.8% of CD8+ T cells, range 1.5–16%). CFSE dilution first became apparent at day 3 after stimulation with peptide and CMV Ag, and the cells divided further during the following period (data not shown). These data indicated that specific peptide stimulation, combined with factors derived from the CD4+ Th cells activated by the CMV Ag, could induce proliferation of the CMV-specific effector cells.

To further address the role of CD4+ Th cells, we depleted CD4+ T cells from PBMCs. As shown in Fig. 3A, this reduced the CFSE dilution of the tetramer+ cells when stimulated with CMV peptide and Ag. Compared with the stimulation of undepleted PBMCs, more CMV-specific cells remained undivided or in the first divisions, confirming that CD4+ T cell-derived factors are indeed involved in driving the proliferation.

The phenotype of the divided CMV-specific CD8+ T cells was determined by flow cytometric analysis. The vast majority of cells switched from CD45RA to CD45R0 expression, but retained the CD27− phenotype (Fig. 1C and data not shown). Interestingly, the chemokine receptor CCR7 reappeared on the cell surface of most of these activated and proliferating CMV-specific cells.

IL-2, IL-15, and IL-21 can support expansion of peptide-stimulated virus-specific effector-type T cells

To test whether induction of CD4+ Th cell activity per se rather than activation of CMV-specific helper cells is necessary to induce expansion of peptide-pulsed effector T cells, PBMCs of a bacillus Calmette-Guérin-vaccinated donor were stimulated with CMV peptide and PPD. As shown in Fig. 2A, in this in vitro setting PPD-specific helpers can support the proliferation of CMV-specific CD8+ T cells.

To identify potential factors involved in the initiation of the proliferation of peptide-stimulated CMV-specific effector cells,
PBMCs were first stimulated with CMV peptide plus CMV Ag with addition of an Ab against CD25. Proliferation of the CMV-specific cells could be partially prevented by blocking the IL-2R α-chain (Fig. 2C), as evidenced by the higher intensity of the CFSE staining and the decrease in the percentage of tetramer+ cells within the CD8+ cells as compared with Fig. 2B (mean CFSE fluorescence intensity, upper left quadrant 88 vs 28). This indicates that at least part of the Ag-induced proliferation is dependent on the actions of IL-2, but that also other cytokines are involved.

We next tested the ability of several cytokines to induce proliferation in combination with CMV peptide. As shown in Fig. 2D–F, strong proliferation was induced when cells were cultured in the presence of peptide and the common γ-chain using cytokines IL-2, IL-15, or IL-21. In the absence of peptide, these cytokines did not induce substantial division in the tetramer-binding population (data not shown), IL-4 and IL-7, which also use the common γ-chain, did not support division of peptide-pulsed CMV-specific T cells, nor did IL-10, IL-12, IFN-γ, or TNF-α.

In contrast to stimulation with peptide and CMV Ag, stimulation of CD4-depleted PBMCs with CMV peptide in combination with IL-2, IL-15, or IL-21 did not differ from cultures with CD4+ T cells present, as shown for IL-15 in Fig. 3B. This indicates that addition of these cytokines is sufficient to support proliferation of the CMV-specific CD8+ T cells.

CMV-specific cells stimulated with peptide and IL-2 switched their surface expression from CD45RA to CD45RO, remained CD27+, and up-regulated CCR7 expression (shown in Fig. 1D). These changes in phenotype are thus comparable with those of cells stimulated with peptide and CMV Ag (Fig. 1C).

Expanded CMV-specific cells retain effector functions

CD8+CD45RA+CD27+ effector T cells use perforin, granzyme B, and CD95L to kill virus-infected cells. As previously shown (8), CMV-specific effector cells, in contrast to stimulation with peptide and CMV Ag, the divided cells still contained these cytotoxic molecules (Fig. 4, C and D) and even obtained a higher perforin content than before stimulation. To test the cytolytic potential of expanded CMV-specific effector cells, cytotoxic assays were performed. Cells that had been stimulated for 5 days with peptide plus IL-2 were added to EBV-transformed target cells loaded with the HLA-A2.1 CMV-specific peptide. Without peptide, no specific lysis of the target cells was found, but CMV-specific cells retained their cytotoxicity toward peptide-pulsed targets (Fig. 5A). On a cellular basis, the percentage of specific lysis did not differ between fresh cells on day 0 and cells that had been stimulated for 5 days. Likewise, effector T cells that had been cultured with peptide in concert with CMV Ag, IL-15, or IL-21 displayed specific cytotoxic potential, and no gross differences between the addition of CMV Ag or cytokines were evident (data not shown). Another key

![FIGURE 3. Depletion of CD8+ T cells reduces proliferation upon peptide and CMV Ag, but not when cytokines are added. Histogram overlays of CFSE dilution of tetramer+ cells stimulated with peptide and CMV Ag (A) or peptide and IL-15 (B). The bold lines are the unstimulated controls; filled histograms represent undepleted PBMCs; and the thin lines are from CD8+ T cell-depleted cultures. Only gated CD8+ CMV tetramer+ T cells are depicted.](image)

![FIGURE 4. CMV-specific CD8+ effector cells contain granzyme B and perforin. Histograms of granzyme B PE and perforin PE staining on fresh cells (day 0, A and B) and after 5 days of stimulation with CMV peptide plus CMV Ag (day 5, C and D). The bold lines represent CD8+ T cells; the bold lines show CD8+ CMV tetramer+ T cells. Both were gated on lymphocytes; for stimulated cultures, blastoid cells also were included. Representative data of three experiments performed are shown.](image)

![FIGURE 5. CMV-specific CD8+ effector cells are cytotoxic. A, Specific lysis of 51Cr-labeled EBV-transformed B cells loaded with CMV peptide. Percentage of specific lysis (y-axis) vs E:T ratio (x-axis). Measured on days 0 (circles) and 5 after stimulation with CMV peptide and IL-2 (triangles). The filled symbols represent the data without peptide loaded; the open symbols indicate the lysis when CMV peptide is loaded on the target cells. Representative data of five experiments performed are shown. B, Dot plots of cells stimulated with PMA-ionomycin before and after 5 days of culture in the presence of CMV peptide and IL-2, gated on CD8+ T cells. Representative flow cytometry is shown from two experiments performed.](image)
function of the effector cells in relation to virus neutralization/elimination is the production of cytokines such as IFN-γ and TNF-α. Indeed, CMV-specific cells that had proliferated upon stimulation with peptide and IL-2 abundantly produced these cytokines after PMA-ionomycin stimulation (Fig. 5B and data not shown). Thus, as judged by both cytotoxic function and cytokine production capacity, expanded CMV-specific cells are functionally competent effector cells.

Discussion

It has been debated whether CD8⁺CD45RA⁺CD27⁻CCR7⁻ T cells (also CD28⁻, LFA-1(±), and perforin) demonstrate immediate effector function with proliferative potential. The notion that CD8⁺CD45RA⁺CCR7⁻ T cells cannot divide and have therefore entered a state of senescence (18) has been based on several lines of evidence. First, CD8⁺CD28⁻ T cells, which largely overlap with CD8⁺CD45RA⁻CD27⁻ T cells (11), have shorter telomere lengths than CD8⁺CD28⁺ cells (19, 20). In this regard, however, did not take into account that a substantial part of the CD8⁺CD27⁺ population consists of naive T cells with relatively long telomeres. Indeed, when CD45RA⁺CD27⁺ and CD45RA⁻CD27⁻ cells were compared, no significant differences in telomere lengths between these two primed sets of CD8⁺ T cells were found (21). Thus, based on telomere status, there is no apparent restriction for further expansion of CD8⁺CD45RA⁺CD27⁻ T cells. Second, Champagne et al. (6) recently showed that CD8⁺CD45RA⁺CCR7⁻ T cells do not divide when a combination of CD3 and CD28 mAbs is used for activation. However, as rightfully pointed out by Wills et al. (14), resting CD8⁺CD45RA⁺CCR7⁻ T cells lack CD28 expression, and an agonistic effect of CD28 mAb on these cells is therefore highly unlikely. Other studies performed in our laboratory have previously shown that proliferation requirements of effector-type CD8⁺CD45RA⁺CCR7⁻ T cells are different from those of the memory type in that the former population only divides in the presence of helper cytokines such as IL-2 and IL-15 (11). This was corroborated in a recent report that demonstrated that CMV-specific T cell populations largely comprised of CD45RA⁺CD28⁺ cells yield an expanded population of Ag-specific cells after 14 days of culture with specific peptide and IL-2 (12, 14). The experiments shown in our present study directly demonstrate that CMV-specific CD8⁺CD45RA⁺CD27⁻CCR7⁻ T cells expand up to six generations and more (based on the CFSE dilution peaks) when stimulated by cognate peptide and Th cell-derived factors. Thus, CD8⁺CD45RA⁺CD27⁻CCR7⁻ T cells are fully differentiated in terms of acquisition of killer cell functions, but not terminally differentiated in regard to having lost the ability to expand. Recent data have shown that the CD4⁺ Th cell-derived cytokine IL-21 limits expansion of IL-15-stimulated NK cells (22). In this way, it could impede the relatively nonspecific activity of NK cells in favor of specific T cells that are generated later during immune responses. Our data seem to be in accordance with this presumed role of IL-21, as expansion of peptide-pulsed effector-type cells is supported by this cytokine.

Phenotypically, reactivated effector-type cells change CD45RA for CD45RO expression, which is in line with the notion that CD45RO is a marker for dividing (or recently divided) T cells (21); this change in phenotype after peptide-specific stimulation was also described by Catalina et al. (23). More striking is the observation that CCR7, a chemokine receptor binding macrophage-inflammatory protein 3β (CCL19) and secondary lymphoid tissue chemokine (CCL21) and directing T cell traffic toward secondary lymphoid organs, is up-regulated on expanded cells. Up-regulation of CCR7 was described earlier upon antigenic stimulation of sorted CD45RA⁺CCR7⁻ cells (6). The biological relevance of the up-regulation is unclear at this moment, but recirculation through spleen or lymph nodes could allow scrutinization of these sites for reactive or reinfesting viruses. Alternatively, migration toward the secondary lymphoid organs could be important to receive competence signals that serve to maintain homeostasis in the effector cell compartment. Because the effector-type cells abundantly produce cytokines, regulatory effects on lymphocytes and APCs could be envisaged. Also, local production of macrophage-inflammatory protein 3β by monocytes and macrophages at sites of infection could direct CCR7⁻ effector cells to their target sites (24).

Phenotypically different virus-specific T cells can be found in latently infected individuals. CD8⁺CD45RA⁺CD27⁻CCR7⁻ effector-memory cells and CD8⁺CD45RA⁻CD27⁻CCR7⁻ effector-type T cells (11, 25). From the analysis of expression of cytolytic mediators and ex vivo cytotoxicity, it is clear that these cells differ in direct effector functions (13, 26, 27). However, the data presented in this study imply that during viral reactivation or re-infection, which is accompanied by the highly epitopes presented by both MHC class I and II to the T cell system, the effector-type cells rapidly change into effector-memory cells and concurrently undergo expansion. This suggests that effector-type T cells not only contribute to the maintenance of latency by directly neutralizing rare virus-expressing cells, but also contribute to immunity by generating vast numbers of new effectors in situations of strong virus reactivation or re-infection.

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