MAJOR ARTICLE

IL-7Rα versus CCR7 and CD45 as Markers of Virus-Specific CD8$^+$ T Cell Differentiation: Contrasting Pictures in Blood and Tonsillar Lymphoid Tissue

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In humans, circulating CD8$^+$ memory T cells to a nonpersistent virus (influenza) lie within CCR7$^+$CD45RA$^-$ central memory, whereas memory to Epstein-Barr virus (EBV) latent, EBV lytic, and cytomegalovirus (CMV) antigens are progressively larger in size and are more biased toward CCR7$^-$CD45RA$^-$ effector memory and CCR7$^-$CD45RA$^+$ terminally differentiated compartments. We found that these populations are also distinguished by progressively lower expression of the interleukin-7 receptor (IL-7Rα) and by lower IL-7 responsiveness; indeed, percentage IL-7Rα–positive values showed a tight inverse correlation with population size. However, these relationships among size, differentiation phenotype, and IL-7Rα status in blood did not hold in tonsillar tissue. In tonsil tissue, although EBV reactivities outnumbered their CMV and influenza counterparts, the distinct CCR7/CD45 isoform signatures of the different virus-specific populations were retained. Moreover, all detectable reactivities showed high levels of IL-7Rα expression. As a discriminator between different virus-specific populations, IL-7Rα therefore appears to be more susceptible to tissue location than the classical CCR7/CD45 markers.

Many viral infections elicit host CD8$^+$ T cell responses that act to bring the infection under control through a capacity to kill virus-infected cells. Thereafter, the immune host retains a population of virus-specific CD8$^+$ T cells in the recirculating memory T cell pool [1, 2]. The factors that are important in maintaining such virus-specific memory are still not fully understood. From work in murine models, it is clear that cytokines of the common γ-chain family, in particular interleukin (IL)–7 and IL-15, are required for the global maintenance and homeostatic proliferation of the memory T cell pool as a whole [3–8]. In mice responding to lymphocytic choriomeningitis virus (LCMV) infection under conditions where the virus is cleared, reexpression of the IL-7 receptor (Rα) on CD8$^+$ T cells has been shown to identify that subset of virus-specific cells within the primary response that are selected to memory [9]. However, for viruses that naturally persist in vivo, chronic antigenic stimulation may also contribute to the maintenance of memory. Indeed, in the LCMV model, under conditions leading to chronic virus infection, it was found that only a fraction of virus-specific CD8$^+$ memory populations later express IL-7Rα [10].

The present work sought to address these questions in the context of human CD8$^+$ T cell responses to different viruses—namely, the nonpersistent influenza virus and 2 persistent herpesviruses, Epstein-Barr virus (EBV) and cytomegalovirus (CMV). These agents are particularly interesting because they elicit memory CD8$^+$ populations that differ both in size, as visualized by tetramer staining of peripheral blood mononuclear

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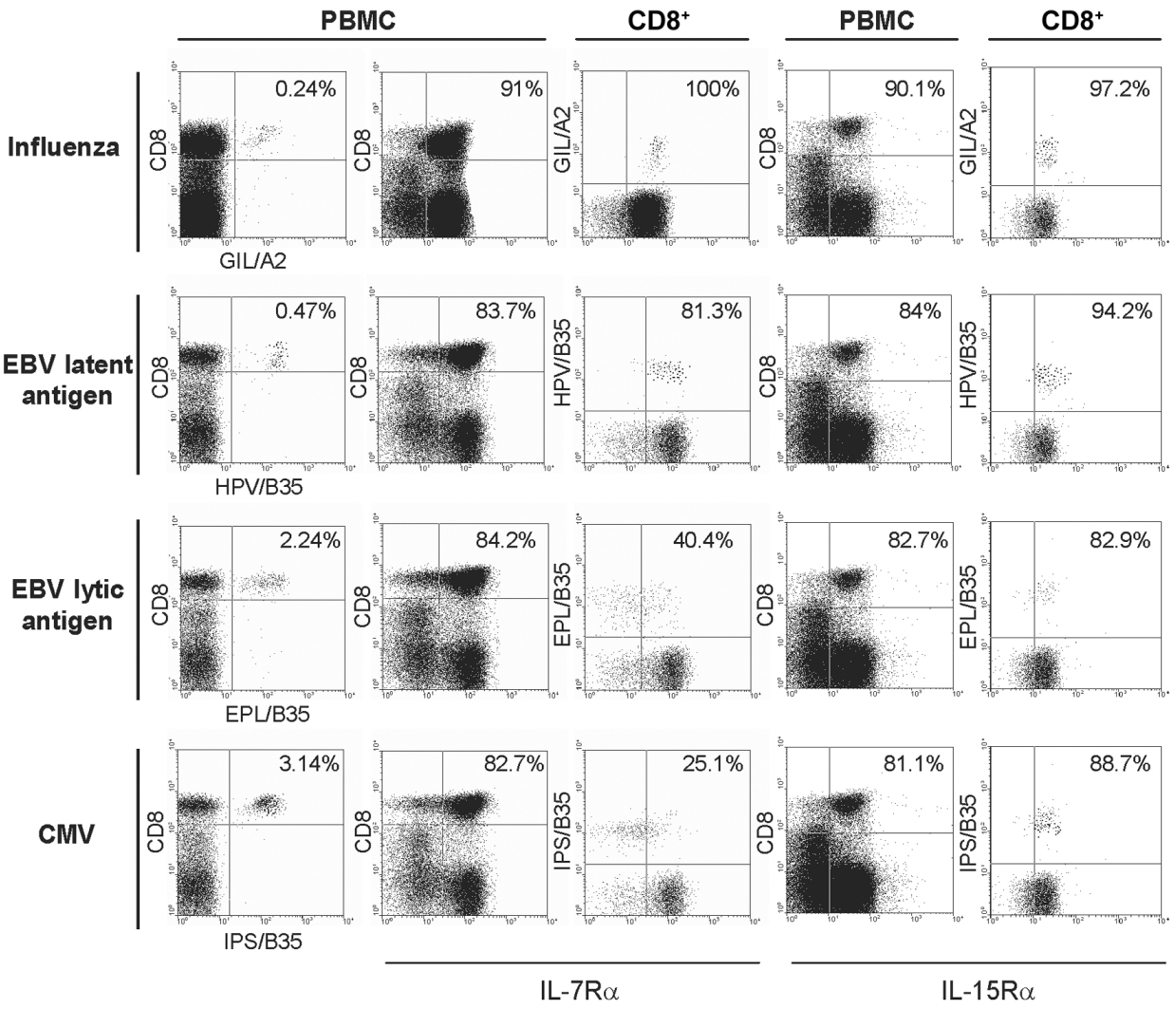

Figure 1. Interleukin (IL)–7–receptor-α (Rα) and IL-15Rα expression on virus-specific CD8$^+$ T cells in blood. Representative staining profiles are shown for memory populations specific for the HLA-A2–restricted influenza epitope GIL in 1 HLA-A2–positive donor and for the HLA-B35–restricted Epstein-Barr virus (EBV) latent HPV, EBV lytic EPL, and cytomegalovirus (CMV) IPS epitopes in a HLA-B35–positive donor. Panels from left to right show CD8/tetramer costaining of peripheral blood mononuclear cells (PBMCs), CD8/IL-7Rα costaining of PBMCs, tetramer/IL-7Rα costaining gated on CD8$^+$ cells, CD8/IL-15Rα costaining of PBMCs, and tetramer/IL-15Rα costaining gated on CD8$^+$ cells. Percentage values refer to the number of double-positive cells expressed as a proportion of all cells positive for the *Y*-axis marker.

cells (PBMCs), and in phenotype, as defined by discriminating markers such as CCR7, CD62L, and the CD45 RA and RO isoforms [11–16]. Indeed, within the EBV system itself there are differences in size and, to some extent, in phenotype between CD8$^+$ T cell memory against the virus latent-cycle epitopes, as opposed to the virus lytic-cycle epitopes [17–19]. Furthermore, in a recent study that monitored individuals recovering from primary EBV infection, the emerging latent- and lytic-cycle memory populations showed different kinetics of IL-7Rα acquisition, and both were significantly delayed, compared with the recovery of IL-7Rα on the CD8$^+$ T cell population as a whole [20].

After a preliminary report indicating that IL-7Rα expression may discriminate between memory populations to persistent versus nonpersistent viruses in humans [21], we examined in more detail the common γ-chain receptor status of influenza, EBV, and CMV-specific CD8 memory populations in blood from healthy adults, in relation to the size and differentiation status of those populations. Then, because our recent findings showed that the size of such populations can be quite different in tonsillar lymphoid tissue than in blood [22], we extended the analysis to determine whether location might influence the cytokine-receptor and differentiation-marker status of these different virus-specific CD8$^+$ populations.

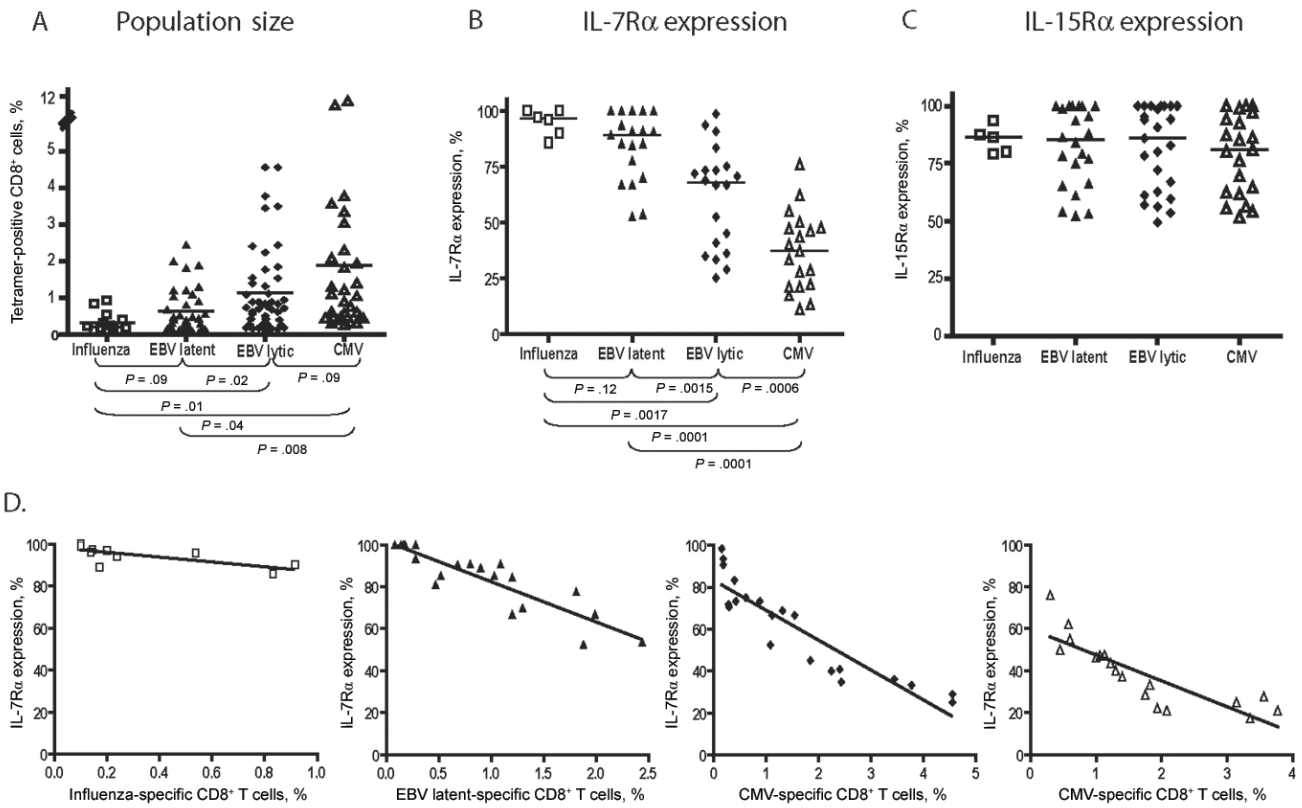

Figure 2. *A,* Scatter plots of the frequencies of tetramer-positive cells in blood expressed as a percentage of the total $CD8^+$ T cell population. Data are shown for reactivities to epitopes from influenza virus *(white squares),* Epstein-Barr virus (EBV) latent *(black triangles),* EBV lytic *(black diamonds),* and cytomegalovirus (CMV) *(white triangles). B–C,* Scatter plots of interleukin (IL)–7–receptor-α (Rα) *(B)* or IL-15Rα *(C)* expression on $CD8^+$ memory populations specific for influenza virus *(white squares),* EBV latent *(black triangles),* EBV lytic *(black diamonds),* and CMV *(white triangles)* epitopes in blood. Each symbol shows the value for a particular epitope in a particular donor, and results are expressed as the percentage of tetramer-positive cells that are IL-7Rα positive. Horizontal lines show the median value for each virus. Statistical analysis was performed with GraphPad Prism software, and the *P* values of significance from the Mann-Whitney *U* test are indicated. *D,* Regression analysis of IL-7Rα positivity versus the percentage of tetramer-positive $CD8^+$ cells in blood. Panels from left to right show correlation for influenza, EBV latent, EBV lytic, and CMV specificities; the coefficients of correlation are $r^2 = 0.54$, 0.79, 0.83, and 0.71, respectively. Statistical analysis was performed with GraphPad Prism software. Note that the 2 highest frequencies in CMV-specific cells have been excluded from the regression graph.

SUBJECTS, MATERIALS, AND METHODS

Donors

Tonsil specimens and matching heparinized blood samples were obtained from 11 patients with no history of EBV-related disease and who provided informed consent, as described elsewhere [22]. Mononuclear cells were isolated by purification over a Lymphoprep gradient (Nycomed) in accordance with the manufacturer's instructions. PBMCs were isolated in a similar manner from peripheral blood specimens obtained at the time of tonsillectomy or isolated from 25 additional healthy adults of known HLA type and with known EBV and CMV serologic results. These experiments were approved by the South Birmingham Health Authority Local Research Ethics Committee.

Phenotypic Analysis

Tetramer staining. The following HLA class I/epitope peptide tetramers were used: influenza epitopes [23] HLA-A2/GILGFVFTL and HLA-B8/ELRSRYWAI; EBV latent epitopes [17, 24, 25] HLA-A2/CLGGLLTMV, HLA-B8/FLRGRAYGL and QAKWRLQTL, and HLA-B35/YPLHEQHGM and HPVGEADYFEY; EBV lytic epitopes [17, 24, 25] HLA-A2/GLCTLVAML and YVLDHLIVV, HLA-B8/RAKFKQLL, and HLA-B35/EPLPQGQLTAY; and CMV epitopes [26] HLA-A2/NLVPMVATV and VLEETSVML, HLA-B8/ELRRKMMYM, ELKRKMIYM, and QIKVRVDMV, and HLA-B35/IPSINVHHY. Peptides were purchased from Alta Biosciences, and phycoerythrin (PE)–conjugated tetramers were produced as described elsewhere [17, 24, 25]. In stainings that included tetramers, cells were first

Table 1. Interleukin (IL) receptor (R) expression on total and antigen-specific CD8+ T cells.

	IL-R–positive cells, % ± SD				
IL-R	CD8+	Influenza	EBV latent	EBV lytic	CMV
IL-2Rα	8.1 ± 3.0	31.6 ± 9.4	6.1 ± 9.6	14.6 ± 12.7	9.2 ± 3.8
IL-4Rα	5.0 ± 3.3	17.5 ± 7.3	8.2 ± 10.6	12.1 ± 26.9	7.3 ± 3.4
IL-7Rα	69.7 ± 13.1	94.5 ± 2.4	89.1 ± 15.4	67.7 ± 22.7	35.5 ± 3.3
IL-15Rα	86.2 ± 6.4	97.6 ± 1.8	94.5 ± 19.7	93.9 ± 19.9	83.4 ± 10.9
IL-21Rα	17.9 ± 3.5	20.6 ± 9.6	25.0 ± 21.3	22.8 ± 14.9	7.1 ± 3.5

NOTE. Nos. indicate the median percentage ± SD of cells expressing IL-2Rα, IL-4Rα, IL-7Rα, IL-15Rα, or IL-21Rα among CD8+ T cells or among influenza-specific, Epstein-Barr virus (EBV) latent–specific, EBV lytic–specific, or cytomegalovirus (CMV)–specific CD8+ T cells in blood. These median values come from the analysis of 25 healthy EBV carriers.

incubated with PE-conjugated tetramer at 37°C for 15 min, and all further steps were conducted on ice.

Panel of anti–human cytokine receptors. Anti–human IL-2Rα, IL-4Rα, and IL-21Rα were purchased from R&D Systems, and staining was detected using secondary fluorescein isothiocyanate (FITC)–conjugated swine anti-goat antibody (Caltag Laboratories).

IL receptor staining. Goat anti–human IL-7Rα antibody (R&D Systems) was detected using FITC-conjugated swine anti–goat IgG antibody (Caltag Laboratories); mouse anti–human IL-15Rα (R&D Systems clone 151307) was detected using FITC-conjugated goat anti–mouse IgG1 (Southern Biotechnology Associates) as described elsewhere [20].

Activation status. In examining mononuclear cell subsets for CD38 expression, tetramer-PE–stained PBMCs were then exposed to the human IL-Rα–specific antibodies listed above, followed by an FITC-conjugated second-step reagent as described above, then washed and stained for 30 min on ice with Tricolour-labeled CD38 monoclonal antibody (MAb; Caltag Laboratories) and energy-coupled dye (ECD)–labeled CD8-specific MAb (Caltag Laboratories).

Differentiation phenotype. Prestained tetramer-PE–stained PBMCs were incubated with Tricolour-labeled CD8 MAbs (Caltag Laboratories) and FITC-labeled MAb to CCR7 (BD Biosciences) plus ECD-labeled anti-CD45RA (Caltag Laboratories) on ice for 30 min, then washed. All stainings were analyzed on an Epics flow cytometer (Beckman Coulter) with appropriate isotypes and color compensation.

Cellular Response to Cytokine Stimulation

PBMCs were exposed to PE-labeled tetramer for 15 min at 37°C in the presence of recombinant IL-7 (R&D Systems) at doses up to 100 ng/mL, then stained using rabbit anti–human phospho–signal transducer and activator of transcription factor (STAT)–5 MAb (Tyr694; Cell Signaling Technology), followed by FITC-conjugated goat anti–rabbit IgG (Southern Biotechnology Associates), in accordance with the manufacturer's instructions, and analyzed as described above. STAT5 phosphorylation analysis was conducted on between a minimum of 100 and a maximum of 1200 tetramer-positive cells, depending on the epitope.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (version 4; GraphPad Prism). Data were compared using a Mann-Whitney *U* test, and significant differences were verified with 95% confidence intervals.

RESULTS

Size and cytokine-receptor status of virus-specific CD8+ memory T cells in blood. Virus-specific CD8+ memory T cells in PBMC preparations from healthy adult donors were identified using PE-labeled HLA/peptide tetramers specific for 2 influenza epitopes, 5 EBV latent epitopes, 5 EBV lytic epitopes, and 6 CMV epitopes, all restricted through HLA A*0201, B*0801, or B*3501. At the same time, the cells were stained with Tricolour-labeled anti-CD8 and FITC-labeled antibodies against individual α-chains of the common γ-chain cytokine receptor family. Figure 1 shows representative results from such an analysis on blood samples, focusing on the IL-7R and IL-15R α-chains. As shown in the left-hand columns, the size of circulating memory populations typically ranged from a low value for influenza-specific, through intermediate values for EBV latent–specific and EBV lytic–specific, to the highest value for CMV-specific memory. The great majority of cells in all 4 virus-specific populations were IL-15Rα positive, as were CD8+ T cells as a whole. By contrast, the percentage of virus-specific memory cells expressing IL-7Rα decreased from 100% for influenza virus, through intermediate levels for EBV latent and lytic populations, to 25% for CMV; this was apparent even though the CD8+ T cell population as a whole was predominantly IL-7Rα positive.

The overall results of such experiments are summarized in figure 2. These data come from a total of 25 healthy donors, most of whom had detectable memory to 3 if not 4 of the antigen specificities studied. This confirms that influenza-specific, EBV latent–specific, EBV lytic–specific, and CMV-specific memory populations are, in that order, progressively larger in size but progressively lower in their percentage of IL-7Rα–positive cells (figure 2*A*–*C*). Indeed, plotting individual values of IL-7Rα positivity versus response size showed remarkably strong correlations for all 4 sets of memory populations studied (figure 2*D*). By contrast, these same populations showed no difference in IL-15Rα status, with the majority of the cells always being IL-15Rα positive. Table 1 summarizes the median percentage positivity shown by the 4 different virus-specific populations for each of the 5 members of the common γ-chain receptor family examined. Expression of IL-2Rα, IL-4Rα, and IL-21Rα was generally restricted to a minority of virus-specific memory cells and of $CD8^+$ T cells as a whole. The only possible exceptions were a tendency for influenza-specific cells to have higher IL-2Rα expression and for CMV-specific memory cells to be particularly low for IL-21Rα.

Size and cytokine-receptor status of virus-specific CD8⁺ memory T cells in tonsils. We then performed the same set of assays on tonsillar mononuclear cell preparations. Typical results from 1 such donor are shown in figure 3. Here, as with almost all tonsil preparations, influenza-specific memory cells were undetectable. By contrast, EBV latent epitope $CD8^+$ T cells were easily detected and were, in fact, more abundant than either EBV lytic epitope reactivities or CMV reactivities. Again, in contrast with the blood, all 3 virus-specific populations were strongly IL-7Rα positive, just as were total tonsillar $CD8^+$ T cells as a whole. Staining for IL-15Rα likewise showed that the large majority of virus-specific and total CD8 T cell populations were receptor positive (data not shown).

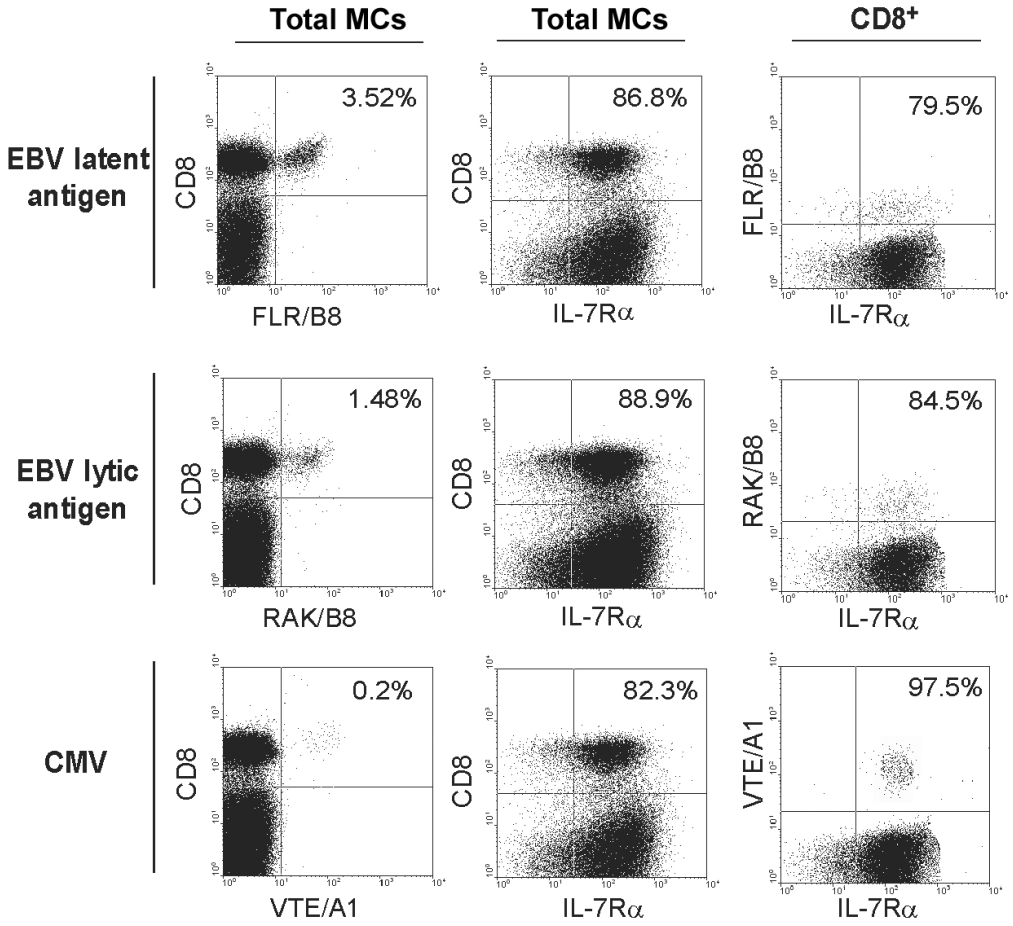

Figure 3. Interleukin (IL)–7–receptor-α (Rα) expression on virus-specific $CD8^+$ T cells in tonsillar lymphoid tissue. Representative staining profiles are shown for memory populations specific for the HLA-B8–restricted Epstein-Barr virus (EBV) latent FLR, EBV lytic RAK, and HLA-A1–restricted cytomegalovirus (CMV) VTE epitopes in the same donor. Panels from left to right show CD8/tetramer costaining of total mononuclear cells (MCs), CD8/IL-7Rα costaining of total MCs, and tetramer/IL-7Rα costaining gated on $CD8^+$ cells. Percentage values refer to the number of double-positive cells expressed as a proportion of all cells positive for the *Y*-axis marker.

The overall results from an analysis of 12 tonsils (all from EBV- and CMV-positive donors, 8 of whom had an HLA type suitable for tetramer staining) are shown in figure 4. These clearly showed that the hierarchy of size distribution, with EBV latent memory as most abundant and CMV as the least abundant, is the opposite of that in the blood. All 3 virus-specific populations had high percentages of IL-7Rα–positive cells, although, interestingly, the CMV memory population was significantly higher than the 2 EBV-specific populations. No differences were seen with respect to IL-15Rα status, which was consistently positive in the large majority of cells (data not shown).

IL-7Rα status and cytokine responsiveness. Given the differences in IL-7Rα profiles apparent from antibody staining experiments, we sought to determine whether such differences truly reflected the ability of the cells to respond to the cytokine itself. For this purpose, we used a fluorescence-based assay of cytokine-induced STAT5 phosphorylation to measure the response of individual cells to a brief exposure to IL-7 in vitro (100 and 1 ng/mL). Figure 5*A* shows typical results from one such assay conducted on matched blood and tonsil cell preparations from a single donor. As illustrated, levels of response (expressed as the percentage of cells stained with a phospho-STAT5–specific MAb) were larger at the higher IL-7 dose. In blood, at both IL-7 doses, responses were highest in influenza-specific memory cells, progressively lower for the 2 EBV-specific populations, and lowest for CMV-specific memory. By contrast, in the tonsil, again at both IL-7 doses, CMV-specific memory responses were higher than those of the 2 EBV-specific populations. Figure 5*B* shows the results of an independent experiment in which the different virus-specific memory T cell responses were compared in blood versus tonsil across a range of IL-7 concentrations, and the results are presented graphically as a dose response. These results confirm the pattern already apparent in figure 5*A*. When the different virus-specific populations were compared, a different hierarchy of responsiveness was observed in blood versus tonsil. Furthermore, these different hierarchies correlated strongly with the different level of IL-7Rα expression seen on virus-specific populations in blood versus tonsil, as already described in figures 2 and 4.

Relationship to the activation/differentiation phenotypes of virus-specific memory cells at the 2 sites. Our earlier studies of virus-specific effector cells activated in vivo during primary EBV or CMV infection showed that activation leads to the transient down-regulation of IL-7Rα [20]. This is illustrated in figure 6*A*, which shows that a highly expanded EBV lytic epitope–specific population in the blood of a patient with acute EBV infectious mononucleosis (IM) is almost uniformly IL-7Rα negative and shows a reciprocal up-regulation of the activation marker CD38. We therefore studied influenza-specific, EBV latent–specific, EBV lytic–specific, and CMV-specific memory-cell populations in blood from healthy donors, to determine whether the differences in their IL-7Rα status was due to differential levels of activation as reflected by CD38 expression. In fact, as shown in figure 6*B*, this was not the case; all 4 populations were predominantly CD38 negative. Furthermore, tonsillar populations of virus-specific cells were all essentially similar to the blood populations in their CD38 profiles (figure 6*C*).

We then turned to another aspect of the memory CD8 phenotype that has been observed to distinguish between different virus-specific reactivities in the blood: the distribution between

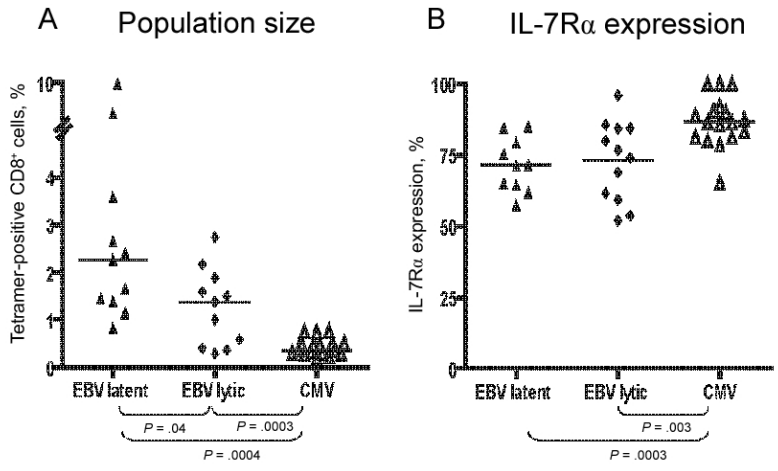

Figure 4. *A,* Scatter plots of the frequencies of tetramer-positive CD8+ T cells in tonsillar lymphoid tissue specific for Epstein-Barr virus (EBV) latent *(black triangles),* EBV lytic *(black diamonds),* and cytomegalovirus (CMV) epitopes *(white triangles)* expressed as in figure 2*A*. *B,* Scatter plots of interleukin (IL)–7–receptor-α (Rα) expression on CD8+ memory populations specific for EBV latent *(black triangles),* EBV lytic *(black diamonds),* and CMV epitopes *(white triangles)* in tonsils expressed as described in figure 2*B*. Horizontal lines show the median value for each virus. Statistical analysis was performed with GraphPad Prism software, and the *P* values of significance from the Mann-Whitney *U* test are indicated.

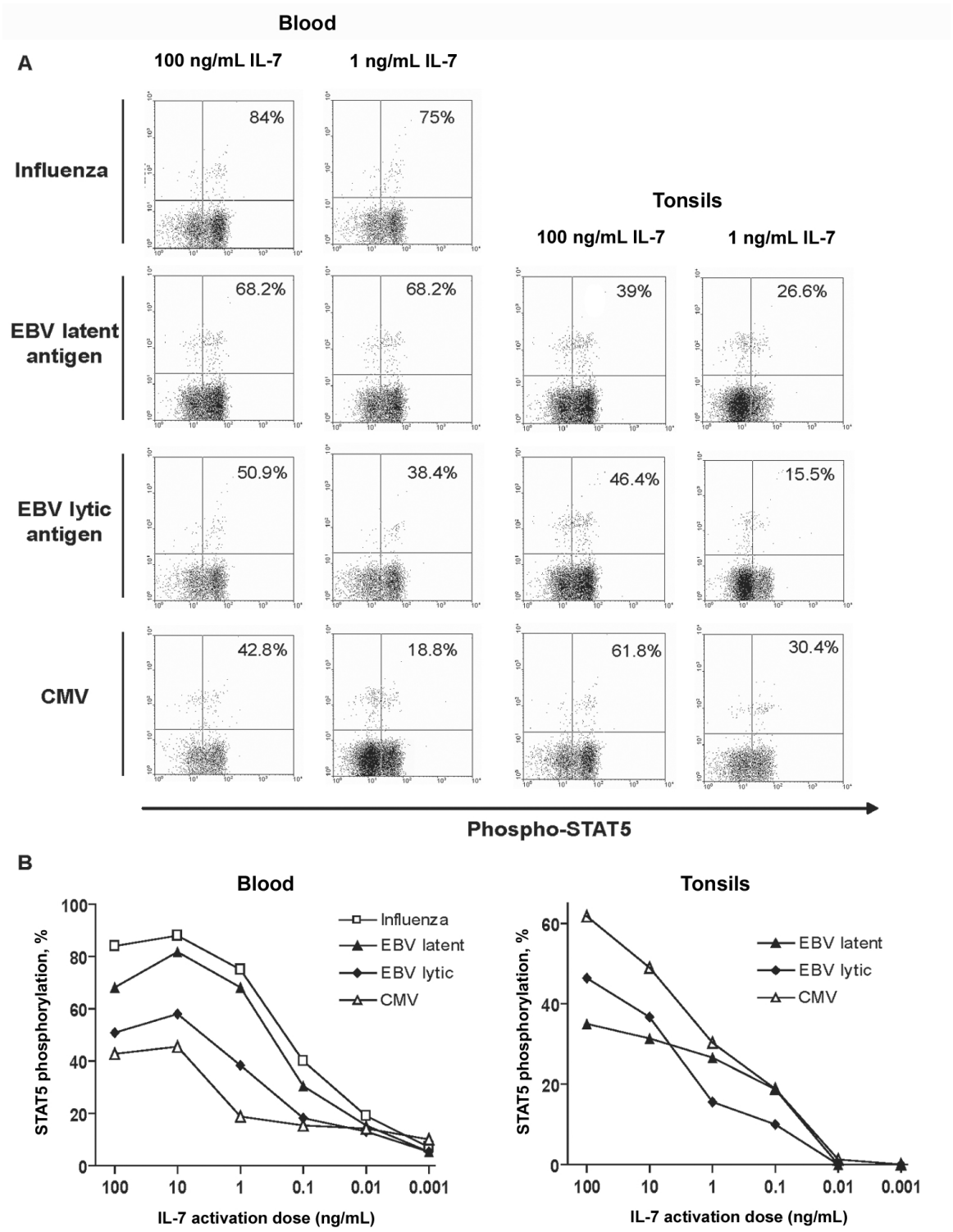

Figure 5. *A,* Representative fluorescence-activated cell-sorting profiles assaying interleukin (IL)–7–induced signal transducer and activator of transcription factor (STAT)–5 phosphorylation in blood *(left)* vs. tonsil *(right)* on virus-specific $CD8^+$ T cells. Levels of phosphorylation after exposure to the IL-7 doses indicated (100 or 1 ng/mL) are shown for influenza-specific, Epstein-Barr virus (EBV) latent–specific, EBV lytic–specific, and cytomegalovirus (CMV)–specific $CD8^+$ memory populations. Percentage values refer to the frequencies of antigen-specific cells that shows phosphorylation of STAT5. *B,* Titration of STAT5 phosphorylation response to IL-7 in memory populations in blood *(left)* for influenza *(white squares),* EBV latent *(black triangles),* EBV lytic *(black diamonds),* and CMV *(white triangles)* epitopes. Similar results are shown for cells isolated from tonsils in the right graph. Results are expressed as the percentage of tetramer-positive cells that showed STAT5 phosphorylation after exposure to the IL-7 doses indicated. This experiment was conducted using the same donor for both peripheral blood mononuclear cells and tonsil preparations; this also showed that absolute level of response (per interleukin-7–receptor-α–positive cells) were slightly lower in tonsillar populations than in blood. The results are representative of several such experiments with different combinations of donors and epitopes.

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 6. *A,* Phenotype and ex vivo activation status of antigen-specific $CD8^+$ T cells. *B,* Ex vivo activation status of epitope-specific $CD8^+$ T cells isolated from the blood of a healthy EBV/cytomegalovirus (CMV)–seropositive donor, assayed by tetramer and CD38 staining. *C,* Ex vivo activation status of epitope-specific $CD8^+$ T cells isolated from tonsillar lymphoid tissue from a healthy EBV/CMV-seropositive donor assayed as in panel B.

the $CCR7^+CD45RA^-$ central memory, the $CCR7^-CD45RA^-$ effector memory, and $CCR7^-CD45RA^+$ terminally differentiated compartments. Figure 7*A* shows typical profiles of the different virus-specific populations in blood. Influenza-specific and EBV latent epitope–specific memory cells tend to be evenly split between central memory and effector memory compartments. In comparison, EBV lytic epitope–specific memory is shifted more toward an effector phenotype, with a few cells even acquiring terminally differentiated status, and CMV-specific memory is very largely within the terminally differentiated compartment, in accordance with previous results [11–16]. We therefore sought to characterize virus-specific memory populations in the tonsil with the same differentiation markers, given that the patterns of IL-7Rα expression on such cells were quite different in blood and tonsil. Despite these contrasting IL-7Rα profiles in the 2 sites, the different virus-specific populations in tonsil retained the same differentiation profiles as in blood. As illustrated in figure 7*B,* the EBV latent epitope–specific population in tonsil remained equally split between central memory and effector memory phenotypes; EBV lytic epitope–specific cells again showed a highest shift toward the effector compartment, and CMV-specific cells were again mostly terminally differentiated.

DISCUSSION

We began these studies in light of observations about patients with IM recovering from primary EBV infection, in whom the recovery of IL-7Rα expression on emerging $CD8^+$ T cell memory was consistently slower and less marked for EBV lytic than for latent epitope reactivities [20]. These differences also correlated with a slower shift of lytic epitope–specific memory toward the $CCR7^+CD45RA^-$ central memory phenotype [18]. Given the work in mouse models that indicated that IL-7Rα recovery was less complete on memory to persistent than to nonpersistent viruses [9, 10], we extended the work to include a nonpersistent infection—influenza—and a second persistent agent—CMV—alongside EBV in the analysis. The present findings, based on the study of 17 viral epitopes in 25 healthy donors, clearly show that there is indeed a relationship between IL-7Rα on memory cells in blood and the type of virus. Influenza-specific memory cells are 80%–100% IL-7Rα positive, and the mean value decreases through EBV latent and lytic reactivities to a minimum of ~30% IL-7Rα for CMV-specific memory. A recent article, which mainly compared influenza- and CMV-induced responses, reported a similar trend [21], in agreement with the broad notion of a difference between nonpersistent and persistent agents. However, our present, more-extensive analysis shows that a striking inverse relationship with response size is superimposed on this pattern. This is apparent not just within the relatively large range of response sizes seen for CMV and for EBV lytic epitope–specific memory but even within the smaller range of influenza responses. Within each group, the larger the individual epitope response, the lower the proportion of memory cells in blood that are IL-7Rα positive.

Studies of primary infection with EBV [20] and CMV [21] have confirmed that antigen-activated $CD8^+$ T cells down-regulate IL-7Rα expression and up-regulate the activation antigens such as CD38. It is therefore conceivable that, in long-term carriers of these viruses, the presence of a fraction of IL-7Rα–negative virus-specific memory cells reflects recent antigen restimulation in vivo [27]. Indeed, this may well be the case in a situation such as chronic HIV carriage, where ongoing virus replication can be intense [28]. However, in the case of both EBV and CMV, we found no evidence of significant virus-specific activation; thus, the great majority of memory cells in the blood were $CD38^-$, and the minority of cells that did express CD38 were no more numerous for memory to these viruses than for influenza-specific memory. However, it is worth noting that, in our earlier studies of patients with IM [20], the reacquisition of IL-7Rα on cells after antigenic stimulation took months to years to complete, long after emerging EBV-specific memory cells had lost their initial CD38 marker of activation. Thus, the failure of circulating memory cells in persistent virus carriers to ever become 100% IL-7Rα positive may reflect occasional restimulation by antigen in vivo. Indeed, the more highly amplified responses, which showed the lowest levels of IL-7Rα expression, may be those most prone to antigen reexposure in vivo, because memory populations immunodominant CMV epitopes do show progressive increases in size over time [23, 29], and a similar effect has been seen under certain circumstances with EBV [23, 30]. The occasional reexposure of individuals to antigenically related influenza viruses over time could likewise serve to boost influenza-specific memory, albeit not as regularly as with a persistent agent. On the basis of the data available in humans so far, we believe that it is premature to draw firm correlations between the IL-7Rα status of circulating memory cells and the persistent/nonpersistent nature of viral infection when the absolute size of the memory population in blood appears to be an important covariable.

In their earlier study, van Leeuwen et al. [21] drew a parallel between IL-7Rα status on virus-specific $CD8^+$ T cells in blood

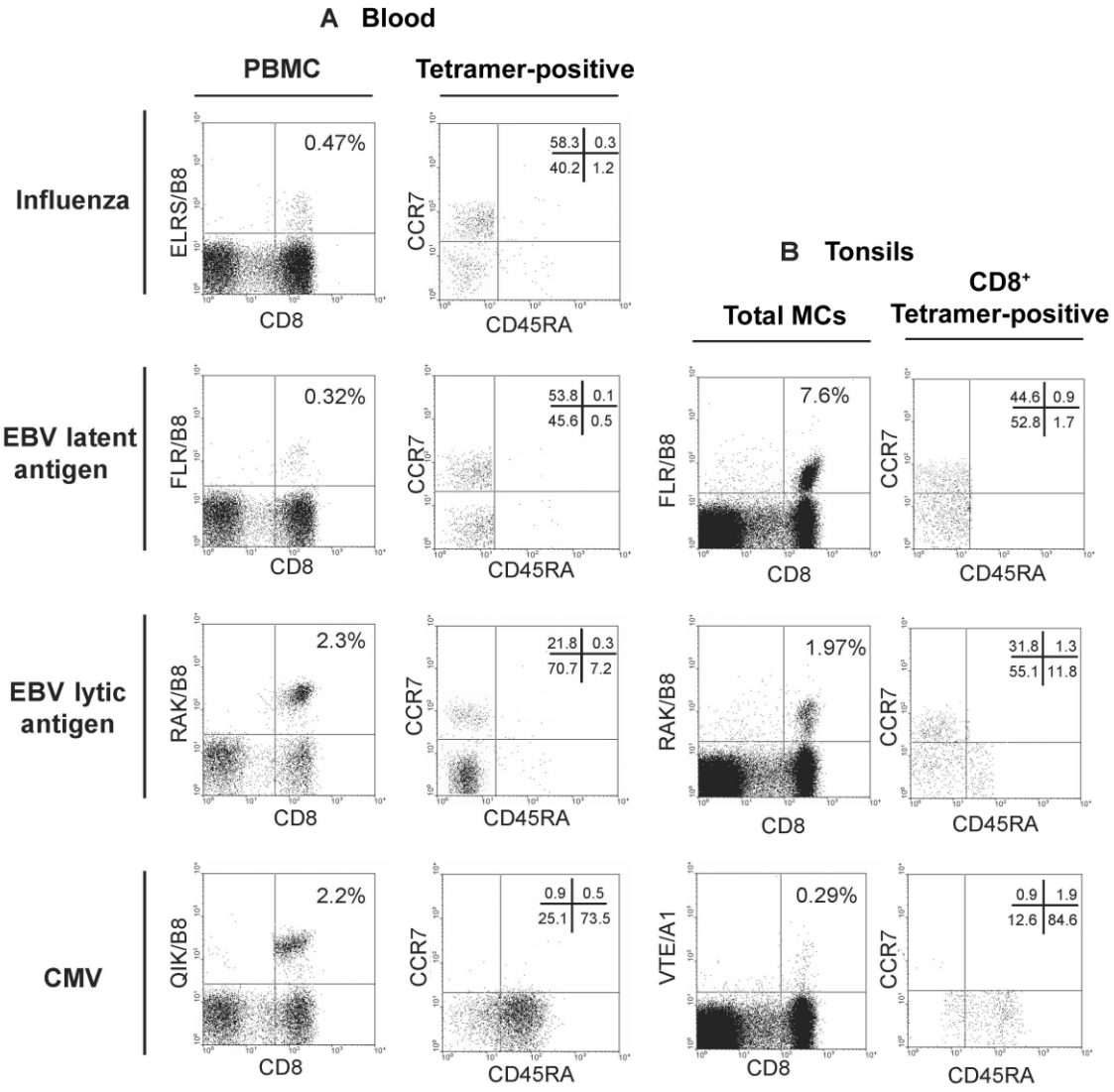

Figure 7. CCR7/CD45RA distribution on antigen-specific CD8$^+$ memory T cells in blood *(A)* vs. tonsil *(B)*. Representative staining profiles are shown for memory populations specific for the HLA-B8–restricted influenza epitope ELRS (exclusively found in blood), for the HLA-B8–restricted Epstein-Barr virus (EBV) latent FLR and EBV lytic RAK epitopes (present in both blood and tonsil), and for the HLA-B8–restricted cytomegalovirus (CMV) QIK (in blood) or HLA-A1-restricted CMV VTE (in tonsil) epitopes. In panels A and B, the left panel shows CD8/tetramer costaining of peripheral blood mononuclear cells (PBMCs) and total mononuclear cells, respectively; the right panel shows CCR7/CD45RA costaining gated on CD8$^+$ tetramer-positive cells. Percentage values refer to the no. of cells positive for each combination of markers. IM, infectious mononucleosis.

and the CD27 costimulation marker. However, this is clearly not the case, because many EBV latent and particularly EBV lytic memory cells are IL-7Rα negative in healthy donors, yet these cells are almost uniformly CD27$^+$ [1, 16, 18]. A closer possible correlate would be IL-7Rα and CD28, but even this correlation does not hold, because CMV-specific memory populations are, on average, 30% IL-7Rα positive in blood, yet almost all of these cells lack CD28 [1, 28]. Our data about circulating virus-specific memory populations are consistent with the finding, made on separated subsets of CD8$^+$ T cells from human blood, that 100% of naive (CCR7$^+$CD45RA$^+$) and central memory (CCR7$^+$CD45RA$^-$) cells and 60%–70% of effector memory (CCR7$^-$CD45RA$^-$) cells but only <20% of terminally differentiated (CCR7$^-$CD45RA$^+$) cells express IL-7Rα (data not shown) [31, 32]. Thus, although in mice IL-7Rα expression has been reported to be a marker that is lost with the transition from central to effector memory on CD8$^+$ T cells [33, 34], there is no absolute correlation between IL-7Rα and other markers of CD8 differentiation in humans.

Arguably the most interesting finding in the present work is the influence of location on the IL-7Rα status of human virus-specific populations in vivo. In this context, most studies to

date in murine model systems have focused on blood and splenic tissue and have reported consistent differences between the high IL-7Rα status of memory to a nonpersistent virus and the lower IL-7Rα status of memory to a persistent virus [9, 10, 33]. However, in a recent study of persistent infection with murine CMV, it was clear that the percentage of virus-specific $CD8^+$ T cells that are IL-7Rα positive varied significantly with tissue site, even when sites where the size of the virus-specific population is similar were compared [33]. Little is known about humans in this regard, except for one recent study in which the IL-7Rα status of different virus-specific memory $CD8^+$ T cells was examined in lungs. For each virus studied, the IL-7Rα profiles seen for memory cells in lungs was broadly similar to that seen in blood, even though the relative representation of respiratory virus-specific memory was enhanced in lungs, compared with EBV- or CMV-specific memory [35]. Our study makes it clear that in at least 1 lymphoid site, the tonsil, the clear differences in IL-7Rα status seen in blood among EBV latent, EBV lytic, and CMV reactivities no longer hold. At that site, all 3 populations are highly IL-7Rα positive, with CMV-specific $CD8^+$ memory in fact showing the high percentage of positivity. As we already reported [22], the relative representation of these populations in tonsillar $CD8^+$ T cells is markedly different from in the blood, with EBV-specific reactivities significantly enriched, CMV reactivities relatively rare, and influenza reactivities undetectable. This may reflect different homing properties imposed on these different populations at the time of priming, as has been reported in several murine studies [36–39]. This could also explain why influenza-specific cells congregate in lungs [35] and EBV-specific cells in oropharyngeal tissues stay present in both cases without obvious signs of local antigenic challenge detectable by activation status.

An important finding was that the EBV latent–specific, EBV lytic–specific, and CMV-specific memory populations in tonsil retained their distinctive CCR7/CD45 differentiation phenotypes, just as is seen in blood. To our knowledge, this extension of the virus-specific memory profile to a site other than blood has not been reported before in human studies. It does suggest that these different signatures, which, at least for the 2 EBV-specific [20] and for CMV-specific [20, 21] populations appear to be imposed soon after primary virus infection, are not just features of circulating cells but will also be apparent at other in vivo sites. By contrast, the IL-7Rα status on $CD8^+$ T cells is clearly more sensitive to local environmental influences, albeit for reasons that are still not clear. Because IL-7Rα expression clearly does correlate with IL-7 responsiveness in our in vitro assays, it may be important that all memory cells become receptive for IL-7–mediated growth/survival signals [7, 9] at particular sites in vivo, and tonsillar tissue is one such site. Our findings clearly highlight the plasticity of IL-7Rα expression at the level of individual $CD8^+$ T cells in vivo and the dangers inherent in using IL-7Rα status as a differentiation marker.

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