Complex Memory T-Cell Phenotypes Revealed by Coexpression of CD62L and CCR7

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Antigen-experienced T cells have been divided into CD62L⁺ CCR7⁺ central memory (T_{CM}) and CD62L[−] CCR7[−] effector memory (T_{EM}) cells. Here, we examined coexpression of CD62L and CCR7 in lymphocytic choriomeningitis virus-specific memory CD8 T cells from both lymphoid and nonlymphoid tissues. Three main points emerged: firstly, memory cells frequently expressed a mixed CD62L[−] CCR7⁺ phenotype that differed from the phenotypes of classical T_{EM} and T_{CM} cells; secondly, T_{CM} cells were not restricted to lymphoid organs but were also present in significant numbers in nonlymphoid tissues; and thirdly, a major shift from a T_{CM} to T_{EM} phenotype was found in memory cells that had been stimulated repetitively with antigen.

Based on expression of CD62L and CCR7, memory T cells have been divided into two main subsets (14): central memory T cells (T_{CM}), expressing both CD62L and CCR7 and representing nonpolarized antigen-experienced cells, and effector memory cells (T_{EM}), lacking CD62L/CCR7 cell surface expression but capable of performing immediate effector cell functions. Studies with mice further demonstrated that in response to antigen, a significant number of T cells leave secondary lymphoid tissues and reside as long-lived memory cells in nonlymphoid tissues (8, 10–12). Memory T cells isolated from nonlymphoid tissues were found to exhibit cytolytic activities and to produce inflammatory cytokines, in contrast to memory cells from secondary lymphoid organs (11, 12). The results of these mouse models fit nicely into the T_{CM}/T_{EM} concept (14), which was based primarily on phenotypic and functional analysis of human memory T cells *in vitro*. However, the *in vivo* CD62L/CCR7 expression patterns of true antigen-specific memory T cells isolated from different lymphoid and nonlymphoid tissues have not yet been characterized in detail.

To address this issue, we used a well-characterized adoptive transfer system with P14 T-cell receptor transgenic cells specific for the major histocompatibility complex class I-restricted GP33 epitope of lymphocytic choriomeningitis virus (LCMV) to generate bona fide memory CD8 T cells *in vivo* (18). Briefly, 10⁵ Thy1.1⁺ P14 T cells were transferred intravenously into C57BL/6 (B6) mice, followed by infection with 200 PFU of LCMV-WE. LCMV infection in this transfer model leads to high viral titers (10⁶ to 10⁷ PFU/g) in the spleen on day 4 after infection. By day 8 postinfection, LCMV is cleared almost completely (<10² PFU/g of tissue) in all organs (19). Tissue distribution and cell surface phenotype of P14 memory cells were determined 7 weeks after LCMV infection by flow cytometry using antibodies specific for Thy1.1 (clone OX-7) and CD62L (clone Mel14) that had been purchased from BD

Pharmingen (San Diego, Calif.). Cell surface expression of CCR7 was determined by a chimeric CCL19-immunoglobulin fusion protein (15). Lymphocytes from lymphoid organs and from perfused liver and lungs were isolated and stained by using standard protocols (13, 15). Collagenase treatment was omitted, since it resulted in partial digestion of CD62L during lymphocyte isolation from liver but not from lung, spleen, and lymph nodes (data not shown). Cells were analyzed on a FACS-Calibur flow cytometer (BD Biosciences). Similar to observations in other viral systems (8, 10, 11), higher frequencies of LCMV-specific P14 memory cells were found in liver and lung than in blood, spleen, and lymph nodes (Fig. 1A).

Examination of P14 memory cells for coexpression of CD62L and CCR7 revealed three major memory T-cell subsets: CD62L⁺ CCR7⁺, CD62L[−] CCR7⁺, and CD62L[−] CCR7[−] cells (Fig. 1B). According to previous definitions (14), CD62L⁺ CCR7⁺ and CD62L[−] CCR7[−] cells were classified as P14 T_{CM} and P14 T_{EM} cells, respectively. The third memory cell population expressing CCR7 in the absence of CD62L was not previously noted and is therefore defined operationally in this study as intermediate memory T cells (T_{IM}). Analysis of P14 memory cells from lymphoid and nonlymphoid tissues revealed the following picture (Fig. 1C): P14 T_{CM} (38% ± 13%), P14 T_{EM} (28 ± 11%), and P14 T_{IM} (25 ± 9%) memory cell populations were present in roughly equal numbers in the blood, P14 T_{CM} cells (60% ± 10%) predominated in the spleen, and P14 T_{CM} (55% ± 9%) and P14 T_{IM} (30% ± 13%) cells represented the two major cell populations in inguinal lymph nodes. In the lung, most of the P14 cells exhibited a T_{EM} phenotype (51% ± 9%), followed by T_{CM} (26% ± 7%) and T_{IM} (16% ± 6%).

The differentiation pathway of T_{CM} and T_{EM} cells is a matter of debate. Sallusto et al. (14) proposed that antigen stimulation leads to T_{CM} cells that can then further differentiate into T_{EM}, whereas data obtained by Wherry et al. (16) with the LCMV mouse model favored the opposite view. The authors of the latter study proposed a conversion of T_{EM} to T_{CM} cells over time after viral clearance. Differentiation of memory T cells may also be influenced by repeated antigenic stimulation (1). To mimic a priming and boosting regimen in our transfer

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FIG. 1. CD62L/CCR7 phenotypes of P14 memory CD8 T cells isolated from lymphoid and nonlymphoid tissues. (A) Distribution of P14 memory cells in B6 recipient mice 7 weeks after adoptive cell transfer and LCMV infection. P14 memory cells were traced with Thy1.1-specific antibodies. The bars represent percentages of Thy1.1⁺ cells of total lymphocytes isolated from the tissues indicated. Values are means $\pm$ standard deviations for five mice. (B) Coexpression of CD62L and CCR7 in P14 memory cells isolated from the tissues indicated. The dot plots show representative CD62L/CCR7 staining gated on Thy1.1⁺ P14 memory cells. (C) Distribution of T_{CM} (CD62L⁺ CCR7⁺), T_{IM} (CD62L⁻ CCR7⁺), and T_{EM} (CD62L⁻ CCR7⁻) phenotypes among P14 memory cells. The bars represent percentages of the indicated memory cell populations in the tissues indicated. Values are means plus standard deviations for five mice. PBL, peripheral blood lymphocytes; ILN, inguinal lymph nodes.

FIG. 2. Repetitive antigen stimulation induces a T_{CM}-to-T_{EM} phenotype shift. (A) Experimental system to generate primary and secondary P14 memory cells. (B) Kinetics of P14 T cells in primary and secondary transfers. Values are mean percentages of P14 T cells of peripheral blood lymphocytes (PBL) of recipient mice at the indicated time points. In primary transfers, spleen cells containing 10⁵ naive P14 T cells were adoptively transferred in B6 mice followed by LCMV infection. In secondary transfers, spleen cells containing 10⁵ primary memory P14 T cells were retransferred into B6 mice followed by a second LCMV challenge infection. (C) Distribution of T_{CM} (CD62L⁺ CCR7⁺), T_{IM} (CD62L⁻ CCR7⁺), and T_{EM} (CD62L⁻ CCR7⁻) phenotypes among primary (black bars) and secondary (white bars) P14 memory cells. The bars represent percentages of T_{CM}, T_{IM}, and T_{EM} cells among primary and secondary P14 memory cells isolated from the tissues indicated. Values are means plus standard deviations for five mice.

model, memory P14 T cells, isolated from the spleen of LCMV immune mice 7 weeks after infection, were retransferred into B6 mice followed by a second LCMV infection (Fig. 2A). This resulted in a strong wave of proliferation of P14 memory cells followed by a decline similar to that observed in primary transfers with naive P14 cells (Fig. 2B). Interestingly, the CD62L/CCR7 phenotype of these secondary P14 memory cells, also isolated 7 weeks postinfection, differed considerably from primary P14 memory T cells. In all organs analyzed, a clear shift from P14 T_{CM} to P14 T_{EM} cells was apparent (Fig. 2C). This was particularly evident in the blood, where the fraction of P14 T_{CM} cells dropped from 38% $\pm$ 13% to 8 $\pm$ 4% and the proportion of P14 T_{EM} cells rose from 28% $\pm$ 11% to 56% $\pm$ 12%. To provide evidence that secondary P14 T_{EM} cells were

FIG. 3. CD62L/CCR7 phenotypes of primary and secondary P14 memory cells. CD62L⁺ CCR7⁺ P14 memory cells from LCMV-immune mice 5 weeks postinfection were purified by cell sorting. These P14 T_{EM} (10⁵) and naive P14 T cells (10⁵) were subsequently transferred into B6 recipient mice, followed by LCMV infection. The dot plots show CD62L/CCR7 expression of P14 cells before cell transfer (top) and at the indicated time point after transfer and infection (bottom). The tissues from which the P14 memory cells were isolated from are indicated on the left. PBL, peripheral blood lymphocytes; ILN, inguinal lymph nodes.

indeed derived from P14 T_{CM} cells, 10⁵ CD62L⁺ CCR7⁺ P14 T cells from primary transfers (5 weeks postinfection) were purified by cell sorting and were retransferred into B6 recipient mice, followed by LCMV infection. The CD62L/CCR7 phenotypes, analyzed 8 weeks after transfer and infection (Fig. 3), were similar to the data shown in Fig. 2C obtained with non-purified P14 memory cells. Comparison of secondary versus primary P14 memory cells again revealed a significant shift from a T_{CM} to a T_{EM} phenotype in all organs analyzed. Hence, our data indicate that repetitive antigen stimulation induces a differentiation from P14 T_{CM} to P14 T_{EM} cells in vivo.

In conclusion, our data demonstrate that memory CD8 T cells frequently express a mixed CD62L⁺ CCR7⁺ phenotype that differs from phenotypes of classical CD62L⁺ CCR7⁺ T_{CM} and CD62L⁺ CCR7⁺ T_{EM} cells. In other words, expression of CD62L and CCR7 in bona fide memory CD8 T cells in vivo overlaps only partially. This point is important, since some groups use expression of CD62L alone to distinguish T_{CM} and T_{EM} cells (5, 16, 17), whereas others use expression of CCR7 (2, 4, 9, 14, 15) for the same purpose. Very recently, coexpression of CD62L and CCR7 in CD4 T cells from influenza virus-infected mice has been described (3). Similar to our data with CD8 T cells, CD4 T cells carrying a mixed CD62L⁺ CCR7⁺ phenotype were also found. However, in contrast to our approach, bona fide antigen-specific memory T cells could not be identified in the latter study. Another important aspect of our report concerns the presence of sizeable numbers of P14 memory T cells in liver and lung that expressed a T_{CM} phenotype. Thus, despite being equipped with two major lymph node homing receptors, CD62L (7) and CCR7 (6), these cells were located in nonlymphoid organs. This result cautions against the exclusive use of cell surface phenotypes to predict tissue localization. Finally, the shift from a T_{CM} to a T_{EM} phenotype in P14 memory cells that had been stimulated twice with antigen indicates that T_{CM}/T_{EM} differentiation pathways could be influenced by a priming/boosting regimen. This result is also relevant for vaccine strategies, since it predicts that repetitive antigen exposure favors T_{EM} differentiation.

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