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Cutting Edge: Migration to Nonlymphoid Tissues Results in Functional Conversion of Central to Effector Memory CD8 T Cells

Amanda L. Marzo,* Hideo Yagita,† and Leo Lefrancçois2*

Memory CD8 T cells, essential for defense against intracellular pathogens, are heterogeneous with respect to phenotype and function. Constitutively lytic effector memory cells primarily reside in nonlymphoid tissues, whereas secondary lymphoid tissues contain functionally quiescent central memory cells. However, the mechanism by which functionally distinct memory populations are maintained is unknown. In this study, we show that resting CD8 memory cells modified their functional abilities upon entry into nonlymphoid tissues, as exemplified by the induction of granzyme B and lytic activity. Contemporaneously, the costimulator CD27 was down-regulated. These findings hold important implications for memory cell lineage development and tissue-specific immunity. The Journal of Immunology, 2007, 179: 36–40.

Seminal studies provide evidence for the existence of two subsets of memory CD8+ T cells, central memory cells (TCM)3 which express the receptors CCR7 and CD62L, allowing efficient homing to lymph nodes, and effector memory cells (TEM) which lack these homing receptors (1, 2) and allow efficient homing to lymph nodes, and effector memory cells primarily reside in nonlymphoid tissues, whereas secondary lymphoid tissues contain functionally quiescent central memory cells. However, the mechanism by which functionally distinct memory populations are maintained is unknown. In this study, we show that resting CD8 memory cells modified their functional abilities upon entry into nonlymphoid tissues, as exemplified by the induction of granzyme B and lytic activity. Contemporaneously, the costimulator CD27 was down-regulated. These findings hold important implications for memory cell lineage development and tissue-specific immunity.

Materials and Methods

Mice and infections

C57BL/6 (Ptprc<sup>-/-</sup> = CD45.2) mice were purchased from The Jackson Laboratory and C57BL/6 (Ptprc<sup>+</sup>) mice from Charles River through the NCI program. B6.SJL-Ptprc<sup>+</sup>Pepcb<sup>+</sup>Tg[TcraTcb]<sup>1100Mjb/J-</sup>B6.129S7<sup>+/−</sup>(CD45.1 OT-I-RAG<sup>−/−</sup>−) were bred at University of Connecticut Health Center. Mice were infected i.v. with VSV-ova (1 × 10<sup>6</sup> pfu) or 1 × 10<sup>6</sup> cfu of LM-ova (15, 16).

Isolation of T cell subsets and adoptive transfer

Lymphocytes were isolated from lymphoid and nonlymphoid tissues as previously described (4). In some experiments, spleen cells were isolated using the same technique that was employed for isolation of nonlymphoid cells. This isolation protocol did not result in alterations of the expression levels of CD27 or CD62L (data not shown). CD27<sup>high</sup>/CD62L<sup>low</sup>, CD27<sup>low</sup>/CD62L<sup>high</sup>, and CD27<sup>high</sup>/CD62L<sup>low</sup> populations were purified by sorting using a FACSVantage SE (BD Biosciences). The purity of sorted samples ranged between 91% and 99% and cell populations were adaptively transferred i.v.

Analysis of intracellular granzyme B expression

For intracellular detection of granzyme B, cells were stained with anti-human granzyme B or an isotype control for 45 min at RT.

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Measurement of cytolytic activity

Cytolytic activity was measured using [3H]sodium chromate-labeled EL4 cells (an H-2b thymoma) with or without the addition of 10 µg/ml of the VSV-N protein-derived peptide RGYVYQGL. Serial dilutions of sorted effector cells were incubated in 96-well round-bottom microtiter plates with 2.5 x 10^5 target cells for 5 h at 37°C. Percent specific lysis was calculated as: 100 x [(cpm released by effectors - cpm released alone)] / [(cpm released by detergent - cpm released alone)].

Results and Discussion

Preferential expression of granzyme B by nonlymphoid memory CD8 T cells correlates with CD27 levels

To determine whether antimicrobial CD8 memory T cells were phenotypically and functionally distinct in different tissues, we compared expression levels of CD27, CD62L, and granzyme B (Fig. 1). Granzye B is involved in lymphocyte-mediated killing of target cells by CTL (17, 18). We have previously shown that unlike tissue-resident cells, splenic memory cells are poorly lytic indicating that there is not a strict correlation between CD62L expression and lytic activity (4). Therefore, we employed CD27 expression in conjunction with CD62L expression to further dissect memory cell subsets, since studies have shown that the CD27^low CD8^+ T cell subset is primarily composed of cells preferentially expressing perforin and exhibiting CTL activity (1, 10, 19). More recently, it has been shown that influenza virus-specific CD27^low T cells are present in the lung after secondary infection and that the loss of CD27 coincides with high granzyme B expression (10). To generate memory cells, CD45.1 OT-I cells were transferred into B6 CD45.2 mice that were then infected i.v. with LM-ova (15, 16), rested for 46 days and secondarily infected with VSV-ova (20). Lymphocytes were isolated 70 days later from the spleen, lung, and liver and the phenotype and granzyme B expression of the Ag-specific T cells was examined. At this time point, small populations of memory CD8 T cells in the spleen and lung expressed CD62L, whereas liver memory cells generally lacked this receptor (Fig. 1). CD27 levels were heterogeneous, with distinct CD27^high and CD27^low memory cell subsets in the spleen and lung whereas liver memory cells were largely CD27^low. Interestingly, granzyme B expression was lowest in splenic memory cells and was substantially higher in lung and liver memory cells (Fig. 1). These data are also consistent with the recent demonstration that CD27 down-regulation correlates with the expression of cytotoxic effector molecules such as granzyme B (10).

FIGURE 1. Phenotype and granzyme B expression of splenic and nonlymphoid memory CD8 T cells. 1 x 10^6 CD45.1 OT-I-RAG^−/− splenocytes were transferred into CD45.2 mice and 1 day later the mice were infected with 1 x 10^6 pfu of LM-ova i.v. and 46 days post infection re-infected with 1 x 10^6 pfu VSV-ova i.v. 70 days later the level of CD62L, CD27 and GrB expression of the OT-I memory CD8 T cells was measured by flow cytometry. Values indicate the mean fluorescent intensity (MFI) of staining for the population. For GrB staining the MFI is shown for the isotype control and test, respectively. Data are representative of 3–4 mice analyzed from two experiments.
Splenic memory cells acquire lytic activity after entry into nonlymphoid tissues

The presence of granzyme B is not an absolute indicator of the ability of a CD8 T cell to mediate target cell lysis since other molecules are also required for CTL activity. Therefore, we measured the direct lytic activity of transferred splenic memory CD8 T cells. Enriched CD8 T cells from spleens of mice previously infected with VSV were adoptively transferred into naïve mice and 4 days later sorted splenic and lung tetramer+/H11001 CD8 T cells were assayed for their CTL capacity. Transferred memory cells isolated from the spleen exhibited low levels of lytic activity (Fig. 3), as did the starting population before transfer (data not shown). In contrast, memory cells obtained from the lung exhibited substantial levels of lytic activity, inducing 10–15% specific lysis at a very low E:T ratio (0.25:1) (Fig. 3). Thus, the induction of granzyme B expression correlated with up-regulation of cytolytic activity in migrating memory CD8 T cells.

Nonlymphoid TEM cells modulate CD27 expression but retain granzyme B following transfer

Our results indicated that TEM cells could convert to functional TCM cells upon entry into nonlymphoid tissue. However, whether this phenomenon represented a “revolving door” through which TEM cells exiting from nonlymphoid tissues reverted to TCM-like cells was unknown. To test this possibility, CD62Llow/CD27lowGrBhigh memory CD8 T cells isolated from the liver were transferred to naïve animals and 4 days later

FIGURE 3. Splenic CD8 Tm acquire lytic activity after entry into nonlymphoid tissues. CD45.2 B6 mice were infected with 1 × 10^5 PFU of VSV-Indiana, rested for 71 days and infected with 1 × 10^6 VSV-New Jersey. Approximately 9 mo after the secondary infection, CD8 T cells were enriched from the spleens and transferred to CD45.1 B6 mice; 4 days posttransfer tetramer+/H11001 CD8 T cells were sorted from the spleen and lungs and set up in a CTL assay. Experiments were performed twice with similar results, and the data are the mean of three mice from one experiment.

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CD27^high cells (A) or CD62L^low/CD27^high cells (B) and transferred to naïve CD45.2 mice (1 × 10^5 and 5 × 10^5, respectively). Four days later lymphocytes were isolated from the spleen, lung, and liver, and the level of CD27, CD62L, and granzyme B expression by the transferred OT-I T cells was determined. C, OT-I transferred mice were infected with LM-ova i.v.; 46 days later mice were re-infected with VSV-ova i.v., and 58 days later splenocytes were isolated, enriched for CD8 T cells, donor cells were sorted, and 8.5 × 10^5 CD27^high cells were transferred to naïve CD45.2 mice. On days 0, 1, and 2, mice were treated with either 250 μg of anti-CD70 (FR70) or control rat IgG. Four days later, lymphocytes were isolated from the spleen, lung and liver, and the level of CD27 was determined on the transferred OT-I T cells. Values indicate the MFI of staining for the population. For GrB staining the MFI is shown for the isotype control and test, respectively. Data are from one representative animal of 1–4 mice analyzed from four experiments (n = 9).
were analyzed. Interestingly, transferred CD62L\textsuperscript{low}/CD27\textsuperscript{low} liver memory cells isolated from the spleen exhibited heightened CD27 levels as compared with their nonlymphoid counterparts in the lung and liver where CD27 expression was similar to that of the cells before transfer (Fig. 4). However, granzyme B levels remained high in transferred memory cells isolated from all tissues. Thus, in this case CD27 expression levels did not apparently correlate with lytic activity. Although at present we do not have an explanation for this finding, given the observed heterogeneity in CD27 expression (Fig. 1), it is perhaps likely that CD27 expression levels may not precisely correlate with granzyme B expression. It is also possible that a period longer than 4 days is required to observe down-regulation of lytic molecules such as granzyme B and this may depend on protein half-life. Alternatively, under normal circumstances lytic memory cells may not exit nonlymphoid tissues in large numbers. Nevertheless, the results again indicated the short-term mutability of the memory cell population with respect to phenotype.

Our data indicated that CD27 but not CD62L levels correlated in most cases with increased levels of granzyme B. Furthermore, CD62L\textsuperscript{low}/CD27\textsuperscript{low}/GrB\textsuperscript{high} memory CD8 T cells appeared to represent terminally differentiated cells whereas both CD62L\textsuperscript{low}/CD27\textsuperscript{high}/GrB\textsuperscript{low} and CD62L\textsuperscript{high}/CD27\textsuperscript{high}/GrB\textsuperscript{low} memory CD8 T cells were functionally flexible. Although it has been suggested that CD27 is lost from CD8 T cells only after repetitive antigenic stimulation (10), we found that down-regulation of CD27 can occur in the absence of Ag, as shown by adoptive transfer of OT-I memory CD8 T cells into naive hosts. However, all memory CD8 T cells we examined expressed CD27 at some level leaving open the possibility that further loss of CD27 may occur following repetitive antigenic stimulation. In addition to modulation of CD27 levels, migration of nonlytic memory CD8 T cells to nonlymphoid tissues triggered the induction of the cytolytic effector molecule, granzyme B and resulted in the induction of lytic activity. These data indicated that memory T cell phenotype and function are not fixed and are dependent on location. Although it remains unclear whether there is a subset of T\textsubscript{EM} cells that preferentially home to nonlymphoid tissues, we would predict that this is not the case as all populations of purified memory T cells homed to the different tissues and up-regulated granzyme B expression. Since cells entering both the lung and liver were functionally modulated, common factors involved in effector molecule regulation must exist in at least these tissues, if not others. It should be noted that our studies utilized secondary memory cells, which may exhibit distinct characteristics as compared with primary memory CD8 T cells. Our original experiments of mouse T\textsubscript{CM} and T\textsubscript{EM} studied primary and secondary memory cells (4) and as reiterated here, showed that splenic primary and secondary memory CD8 T cells contain at least a subpopulation of cells with a T\textsubscript{EM} phenotype that display negligible levels of lytic activity. Recent reports indicate that lytic activity, granzyme B expression and protective capacity of memory CD8 T cells increase with the number of booster immunizations (25, 26). Nonetheless, a subset of T\textsubscript{CM} is apparent after multiple immunizations, particularly when the lack of CD62L expression is not used as criteria to identify T\textsubscript{EM}. The length of time after immunization is another important criteria to consider when assessing memory CD8 T cells since phenotype and perhaps function can change over time as a result of population dynamics (26–28). In any case, our data favor the hypothesis that one consequence of traversing the endothelium to enter parenchymal tissues is induction of lytic activity. This phenomenon may be regulated by T cell interactions with chemokines and adhesion molecules that are necessary for extravasation of memory T cells from blood vessels to extralymphoid sites. A pathway such as this could represent a regulatory mechanism for ensuring optimal activity of memory T cells in the appropriate locale.

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Disclosures
The authors have no financial conflict of interest.

References

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\caption{Nonlymphoid T\textsubscript{EM} cells modulate CD27 expression but retain granzyme B following transfer. OT-I transferred CD45.2 B6 mice were infected with LM-ova and 48 days later were infected with VSV-ova i.v.; 97 days later, CD62L\textsuperscript{low} liver memory T cells were sorted and 1 × 10\textsuperscript{5} OT-I cells were transferred to naïve CD45.2 B6 recipients, and 4 days posttransfer CD27, CD62L, and granzyme B expression of OT-I cells from the spleen, lung, and liver was determined. Values indicate the MFI for staining with the population. For GrB staining, the MFI is shown for the isotype control and test, respectively. Data are from one representative animal of two mice analyzed from two experiments.}
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