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Cutting Edge: The Transcription Factor Eomesodermin Enables CD8+ T Cells To Compete for the Memory Cell Niche

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CD8+ T cells responding to intracellular infection give rise to cellular progeny that become terminally differentiated effector cells and self-renewing memory cells. T-bet and eomesodermin (Eomes) are key transcription factors of cytotoxic lymphocyte lineages. We show in this study that CD8+ T cells lacking Eomes compete poorly in contributing to the pool of Ag-specific central memory cells. Eomes-deficient CD8+ T cells undergo primary clonal expansion but are defective in long-term survival, populating the bone marrow niche and re-expanding postrechallenge. The phenotype of Eomes-deficient CD8+ T cells supports the hypothesis that T-bet and Eomes can act redundantly to induce effector functions, but can also act to reciprocally promote terminal differentiation versus self-renewal of Ag-specific memory cells. The Journal of Immunology, 2010, 185: 4988–4992.

Memory CD8+ T cells can be categorized into at least two groups, effector memory and central memory, distinguishable by cell surface marker expression, anatomic location, and functional properties (1, 2). Effector memory CD8+ T cells are more prevalent in peripheral, non-lymphoid tissues, rapidly exert effector functions upon Ag encounter, and have limited proliferative capacity. Central memory cells retain greater capacity for secondary re-expansion and greater long-term persistence, enabled by efficient homeostatic self-renewal. Central memory CD8+ T cells are more prevalent in lymphoid tissues including the bone marrow, spleen, and lymph nodes. Of these tissues, the bone marrow is thought to provide a niche that supports homeostatic self-renewal and acts as a reservoir for memory CD8+ T cells (3–6).

Prior work supports critical roles for two members of the T-box factor family, T-bet and eomesodermin (Eomes), in the formation of CD8+ T cell effector and memory subsets (7–9). The observation of enhanced central-memory differentiation in CD8+ T cells lacking T-bet suggests that T-box factors may serve as regulators of CD8+ T cell propensity for terminal effector differentiation versus persistence as long-lived memory cells (10, 11). In this study, we have evaluated terminal differentiation versus memory cell development in CD8+ T cells lacking Eomes. We observe diminished capacity to compete for the Ag-specific memory compartment in CD8+ T cells lacking Eomes. Eomes-deficient memory CD8+ T cells have defects in long-term persistence and secondary expansion postrechallenge, two hallmark properties of central memory CD8+ T cells. We further observe diminished ability to compete effectively for the bone marrow memory niche in memory CD8+ T cells lacking Eomes. These results suggest that Eomes confers competitive fitness to memory cells and support a role for Eomes in promoting persistence as long-lived memory versus terminal effector differentiation of Ag-specific CD8+ T cells.

Materials and Methods

Mice
Mice were used in accordance with the University of Pennsylvania Institutional Animal Care and Use Guidelines (Philadelphia, PA). C57BL/6 mice, P14 TCR-transgenic mice, Tbx21−/− (T-bet knockout [KO]) mice, Eomesfl/fl CD4−Cre (Eomes KO) mice (12), Thy1.1 mice, CD45.1 mice, and RAG1−/− mice were backcrossed to C57BL/6 for at least 10 generations. For adoptive transfer experiments, 5 × 106 Thy1.1 P14 CD8+ T cells were mixed with 5 × 104 Eomes KO P14 CD8+ T cells transferred i.v. into CD45.1 recipients, with viral infection the following day.

Infections
Mice were infected with 2 × 105 PFU (initial challenge) or 1 × 106 PFU (rechallenge) lymphocytic choriomeningitis virus (LCMV) Armstrong strain by i.p. injection. For rechallenges, 5 × 105 Listeria monocytogenes-expressing GP35–43 (GP33) were injected i.v.

RAG1−/− bone marrow chimeras
Recipient RAG1−/− mice were subjected to sublethal irradiation (400 rad) and injected i.v. with 5 × 105 Thy1.1 bone marrow cells mixed with 5 × 106 Eomes KO bone marrow cells harvested on the same day. Eight to 10 wk posttransplant, peripheral blood from each recipient was analyzed for presence and relative numbers of CD8+ T cells, CD4+ T cells, and B cells derived from each background.

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The online version of this article contains supplemental material.

Abbreviations used in this paper: Eomes, eomesodermin; GP33, GP35–43; KO, knock-out; LCMV, lymphocytic choriomeningitis virus; NP93, NP90–96; WT, wild-type.

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Flow cytometry and T cell stimulation

Surface staining, peptide stimulations, intracellular cytokine staining, H-2Dβ, GP33, and NP396–404 (NP396) tetramer staining and flow cytometry were done as described (12). Abs used for flow cytometry were purchased from BD Biosciences (CD44, CD62L, CD122, CD127, CD27, IFN-γ, KLRE1, Thy1.1, and BrdU; San Jose, CA) or eBioscience (Eomes, CXCR4, integrin α4, and integrin B1; San Diego, CA).

BrdU incorporation

Mice received 0.2 ml PBS containing 2 mg BdU by i.p. injection daily for 7 d preanalysis.

Quantitative real-time PCR

The quantitative real-time PCR primer and probe set used for hypoxanthine phosphoribosyltransferase was previously described (10). Presynthesized TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) were used to amplify CXCR4, CXCR3, and Bcl-2. Sample gene values are expressed relative to hypoxanthine phosphoribosyltransferase, with the lowest value standardized at 1.

Results and Discussion

Diminished central memory CD8+ T cell population in the absence of Eomes

To evaluate the expression of Eomes at the single-cell level in CD8+ T cells responding to infection, we infected C57BL/6 mice with LCMV Armstrong and analyzed the expression of Eomes using intracellular flow cytometry in LCMV-specific CD8+ T cells. Specificity of Eomes staining was validated using Eomes KO cells (Supplemental Fig. 1). Compared to CD8+ T cells 8 d after LCMV infection, the vast majority of which are effector cells, LCMV-specific memory CD8+ cells 90 d after infection express higher levels of Eomes (Fig. 1A). Our results support a correlation between Eomes protein expression and preservation as LCMV-specific memory.

We next examined the effector memory (CD44hiCD62Llo) and central memory (CD44hiCD62Lhi) CD8+ T cell compartments in spleens of 6–12-mo-old WT and Eomes KO mice 60 d postinfection with LCMV. Data in A and B are representative of three independent experiments. C, GP33-specific central memory CD8+ T cells in spleens of WT versus Eomes KO mice 60 d postinfection with LCMV. Numbers within plots refer to percentage of cells in the respective quadrant. Data are representative of three independent experiments. D, Central memory (CD62LhiCD44hi), effector memory (CD62LloCD44hi), and naive (CD62LloCD44lo) CD8+ T cell compartments in spleens of 6- to 12-mo-old naive, noninfected WT, T-bet KO, or Eomes KO mice. Numbers within plots refer to percentage of cells in the respective quadrant. Data are representative of three independent experiments. E, Listeria monocytogenes-GP33 burden in liver or spleen 3 d postinfection with 5 × 105 Listeria monocytogenes-GP33 organisms in nonimmune mice (nonimmune WT), immunized (by LCMV infection 100 d prior) WT mice (immune WT), and immunized Eomes KO mice (immune KO) with three mice per group. Data are representative of two independent experiments.

Nonetheless, the data are compatible with a role for Eomes in promoting central memory-like cell persistence.

Eomes-deficient CD8+ T cells contribute poorly to the memory cell pool when competing with normal cells

The relative defect in LCMV-specific CD8+ T cell memory in Eomes KO mice indicates that although Eomes is not essential in memory development, its expression in individual CD8+...
Defective memory CD8+ T cell persistence and re-expansion in the absence of Eomes in competitive models. A. Serial flow cytometric analysis of peripheral blood from Eomes KO/WT bone marrow chimeras, showing CD8+ GP33+ and CD8+ NP396+ cells derived from Eomes KO bone marrow or WT bone marrow, as labeled, versus time in relation to initial LCMV infection. Data are representative of three independent experiments. B. Ratio of WT to Eomes KO CD8+ GP33+ T cells (top panel) and IFN-γ+ CD8+ T cells (bottom panel) in the spleen of an Eomes KO/WT bone marrow chimera spleen 5 d postrechallenge with LCMV (65 d after primary infection). Numbers within quadrants represent percentage of cells within that quadrant. Data are representative of five independent experiments.

C. Relative number of Eomes KO (CD8+ Thy1.2+CD45.1+) and WT (CD8+ Thy1.2+CD45.1+) P14 cells in the blood of recipients 8 d or 45 d after primary LCMV infection. Data are mean ± SEM and are derived from four chimeric mice in each of three independent experiments. D. Ratio of WT versus Eomes KO CD8+ GP33+ cells in Eomes KO/WT bone marrow chimeras 60 d after LCMV infection. Data presented as mean and SEM from four individual chimeras. E. Ratio of P14 cells in spleen relative to bone marrow 8 d after LCMV infection for both WT and Eomes KO populations in a competitive adoptive transfer setting. Data are representative of seven cotransfer recipients over two independent experiments. *p < 0.01 versus blood; **p < 0.05 versus lymph node or spleen. F. Competitive adoptive transfer setting. Data are representative of seven cotransfer recipients over two independent experiments. F. Relative number of Eomes KO (CD8+ Thy1.2+CD45.1+) and WT (CD8+ Thy1.2+CD45.1+) P14 cells in the blood of recipients 8 d or 45 d after primary LCMV infection. Data are mean ± SEM and are derived from four chimeric mice in each of three independent experiments. G. Ratio of WT versus Eomes KO GP33+ CD8+ T cells in Eomes KO/WT bone marrow chimeras 60 d after LCMV infection. Data presented as mean and SEM from four individual chimeras. H. Eomes expression in short-lived effector (KLRG1hi CD127lo CD8+ GP33+) and memory precursor (KLRG1hi CD127hi CD8+ GP33+) populations in spleens from mice 8 d after LCMV infection. Data are mean ± SEM and are derived from four mice per experiment. I. Eomes expression in short-lived effector (KLRG1hi CD127lo CD8+ GP33+) and memory precursor (KLRG1hi CD127hi CD8+ GP33+) populations in spleens from mice 8 d after LCMV infection. Data are mean ± SEM and are derived from four mice per experiment. J. Competitive adoptive transfer setting 8 d after LCMV infection. Data are representative of two independent experiments with a total of seven cotransfer recipients. *p < 0.01 versus blood; **p < 0.05 versus lymph node or spleen. Student two-tailed t test.
GP33-specific) TCR-transgenic CD8\(^+\) T cells (hereafter referred to as WT P14 and marked by Thy1.1) with an equal number of Eomes KO P14 CD8\(^+\) T cells (marked by Thy1.2) and injected the combination into CD45.1 non–TCR-transgenic recipients. We infected recipient animals with LCMV and followed the relative abundance of Eomes KO P14 and WT P14 CD8\(^+\) T cells. We found no consistent differences between Eomes KO and WT effector CD8\(^+\) T cells expansion 8 d postinfection (Fig. 2C). After viral clearance (day 45), the relative percentage of memory Eomes KO P14 cells declined precipitously (Fig. 2C). We analyzed the relative prevalence of Eomes KO versus WT GP33-specific memory cells in the blood, lymph nodes, spleen, and bone marrow of Eomes KO/WT bone marrow chimeric mice 60 d postinfection with LCMV (Fig. 2D). We observed a consistent hierarchy among different tissues, with the least amount of skewing in the blood, modestly increased skewing in the spleen and lymph nodes, and markedly higher skewing in the bone marrow (Fig. 2D). Although much of the data shown are derived from blood, similar observations were made in almost all lymphoid organs (not shown). In the setting of acute infection, we observed a 2.3-fold defect in bone marrow localization in Eomes KO P14 cells 8 d after LCMV infection in the competitive adoptive transfer model (Fig. 2E), suggesting a role for Eomes in bone marrow localization in both effector and memory CD8\(^+\) T cells.

Upon reinfection of immune mice 45 d after initial infection, WT P14 cells underwent re-expansion, whereas Eomes KO P14 cells underwent virtually no expansion and remained barely detectable (Fig. 2F). The defect in Eomes KO memory CD8\(^+\) T cell re-expansion when competing with WT cells was more severe than differences observed in nonchimeric Eomes KO and control mice rechallenged with LCMV 90 d after initial infection (Fig. 2F, 2G). The results suggest that Eomes enables CD8\(^+\) T cells to compete for niches and signals that promote memory differentiation rather than being an absolute regulator of memory differentiation.

The results raise the possibility of a relationship between Eomes expression and the development of memory-precursor (KLRG1\(^hi\) CD127\(^lo\)) CD8\(^+\) T cells (11). In the setting of acute LCMV infection, we observed similar levels of Eomes in memory precursors (KLRG1\(^hi\) CD127\(^hi\)) and short-lived effector cells (KLRG1\(^hi\) CD127\(^hi\)) (Fig. 2H). Similarly, 8 d postinfection, short-lived effector and memory-precursor population frequencies do not significantly differ within Eomes-deficient and -sufficient populations in a competitive setting (Fig. 2I). Several months after viral clearance, we observed significantly higher levels of Eomes in central memory (CD44\(^hi\) CD62L\(^lo\)) relative to effector memory (CD44\(^hi\) CD62L\(^hi\)) CD8\(^+\) T cells (Fig. 2J). Taken together, these data do not support a role for Eomes in promoting the memory-precursor fate. Instead, the results provide evidence that Eomes is involved in bone marrow localization, long-term persistence, and re-expansion capacity of memory cells.

Defective population of the bone marrow niche by Eomes KO memory CD8\(^+\) T cells

The different ratios of WT to Eomes KO memory CD8\(^+\) T cells in different lymphoid tissues raise possibilities of altered bone marrow survival, proliferation, or trafficking in CD8\(^+\) T cells lacking Eomes. Molecules associated with memory T cell bone marrow localization include the chemokine receptor CXCR4 and VLA-4, which is composed of integrin \(\alpha 4\) and integrin \(\beta 1\) (5). We observed diminished expression of CXCR4, but neither integrin \(\alpha 4\) nor integrin \(\beta 1\), in Eomes KO GP33-specific memory CD8\(^+\) T cells from Eomes/WT bone marrow chimeric mice compared with WT 60 d after LCMV infection (Fig. 3A). Sorted Eomes KO central memory (CD44\(^hi\)CD62L\(^lo\)) CD8\(^+\) T cells from Eomes/WT bone marrow chimeric mice 60 d after LCMV infection were found to have less CXCR4 and CXCR3 mRNA compared with their WT counterparts (Fig. 3B). Currently, there does not appear to be a known role for CXCR3 in bone marrow homing.

Diminished persistence of Eomes KO memory CD8\(^+\) T cells could be related to defects in homeostatic proliferation, survival, or both. The highest rate of homeostatic proliferation of memory CD8\(^+\) T cells is found in the bone marrow, and in bone marrow memory CD8\(^+\) T cells, we observed a modest reduction in proliferation in Eomes KO cells compared with WT as measured by BrdU incorporation (Fig. 3C). In addition to their proliferation defect, a survival disadvantage is suggested by our observation of reduced expression of Bcl-2 mRNA in Eomes KO memory CD8\(^+\) T cells (Fig. 3D).

Proliferation and survival of memory CD8\(^+\) T cells are supported by cytokine signals, including IL-15 and IL-7, as well as CD27–CD70 interactions (1). We found a modest but reproducible defect in CD122 (IL-15R\(\beta\)) expression but no deficit in expression of CD127 (IL-7R\(\alpha\)) or CD27 on Eomes KO memory CD8\(^+\) T cells (Fig. 3E, Supplemental Fig. 2). These data suggest that reduced proliferation and survival might both be involved in the defective persistence of Eomes KO memory CD8\(^+\) T cells.

In summary, we provide evidence that CD8\(^+\) T cells lacking Eomes are less fit for preservation as memory and population

![FIGURE 3. Defective bone marrow localization and proliferation Eomes KO memory CD8\(^+\) T cells. A, CXCR4, integrin \(\alpha 4\), and integrin \(\beta 1\) expression on CD8\(^+\) GP33\(^+\) T cells from spleens of Eomes KO/WT bone marrow chimeras 60 d after LCMV infection. B, Quantitative RT-PCR of CXCR4 or CXCR3 mRNA from WT versus Eomes KO central memory (CD44\(^hi\) CD62L\(^lo\)) CD8\(^+\) T cells sorted from an individual Eomes KO/WT bone marrow chimeric 60 d postinfection with LCMV. Data are representative of two independent experiments. C, BrdU uptake in Eomes KO versus WT CD8\(^+\) GP33\(^+\) T cells from the bone marrow of Eomes KO/WT bone marrow chimeras 60 d after LCMV infection. Data are derived from four chimeric animals. D, Quantitative RT-PCR of Bcl-2 mRNA as in B. E, CD122, CD127, and CD27 expression on CD8\(^+\) GP33\(^+\) T cells from spleens of WT and Eomes KO mice 60 d postinfection with LCMV. Data are representative of three independent experiments.](http://www.jimmunol.org/)
of the bone marrow memory niche. These observations are in keeping with a model in which the relative expression levels of Eomes and T-bet may contribute to the adoption of a memory fate versus terminal effector differentiation in CD8+ T cells. Given the ability to manipulate the relative expression of T-bet and Eomes with agents including rapamycin and IL-12 (13, 14), the data provide rationale for novel approaches to generating long-lasting immunity.

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Disclosures
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