A major histocompatibility complex class I–dependent subset of memory phenotype CD8+ cells

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Most memory phenotype (MP) CD44hi CD8+ cells are resting interleukin (IL)-15–dependent cells characterized by high expression of the IL-2/IL-15 receptor β (CD122). However, some MP CD8+ cells have a CD122lo phenotype and are IL-15 independent. Here, evidence is presented that the CD122lo subset of MP CD8+ cells is controlled largely by major histocompatibility complex (MHC) class I molecules. Many of these cells surface markers typical of recently activated T cells (CD62Llo, CD69hi, CD43hi, and CD127lo) and show a high rate of background proliferation. Cells with this phenotype are highly enriched in common γ chain–deficient mice and absent from MHC-I−/− mice. Unlike CD122hi CD8+ cells, CD122lo MP CD8+ cells survive poorly after transfer to MHC-I−/− hosts and cease to proliferate. Although distinctly different from typical antigen–specific memory cells, CD122lo MP CD8+ cells closely resemble the antigen–dependent memory CD8+ cells found in chronic viral infections.
“background” proliferation (turnover) and survival (13). In marked contrast, the remaining ∼30–40% of MP CD8+ cells are CD122lo and IL-15 independent. As a consequence, CD122lo MP CD8+ cells account for nearly all of the remaining MP CD8+ cells in IL-15−/− mice. These cells are unaffected by IFN-induced (IL-15–mediated) bystander proliferation and survive well upon adoptive transfer to IL-15−/− mice (13). We decided to investigate the factor(s) responsible for the survival and turnover of the CD122lo subset of MP CD8+ cells.

Here, we demonstrate that the majority of CD122lo MP CD8+ cells are a unique population with the phenotype of activated cells. These cells account for most of the residual CD8+ cells in γc−/− mice, implying lack of dependence on γc cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) for their survival and turnover. Significantly, CD122lo MP CD8+ cells disappear rapidly and cease to proliferate upon transfer to MHC-I−/− hosts. Hence, unlike typical IL-15–dependent CD122hi cells, the CD122lo subset of MP CD8+ cells is largely cytokine independent and kept alive through continuous TCR contact with MHC-I ligands.

**RESULTS**

**Phenotypic features of MP CD8+ subsets**

Normal animals accumulate CD44hi MP CD4+ and CD8+ cells with increasing age, presumably as a result of lifetime exposure to various environmental antigens or self-antigens. In young (2–3-mo-old) C57BL/6 (B6) mice, MP T cells account for ∼10% of the T cells in the LN and ∼15% of splenic T cells (Fig. 1 A). Although CD44 levels are comparable on CD122hi CD44hi MP cells and naive CD44lo CD8+ cells, proliferation of CD44lo cells is restricted to BrdU intermediate cells, thereby resembling TCR-activated T cells (Fig. 1 C). Also, proliferation, as measured by incorporation of the DNA precursor BrdU, given for 3 or 7 d (Fig. 1 C). Note that proliferation is restricted to BrdU intermediate cells, which are recent thymic emigrants that incorporated a low level of BrdU in the thymus before export (22). Despite their similar surface markers, CD122hi MP CD8+ cells and naive CD8+ cells were clearly different with regard to Bcl-2 expression and IFN-γ production. Thus, relative to naive CD8+ cells, CD122hi MP CD8+ cells showed two- to three-fold higher levels of Bcl-2 (Fig. 1 D) and >10-fold higher production of IFN-γ upon in vitro stimulation (Fig. 1 E).

The properties of the CD122hi subset of CD8+ MP cells were quite different. Thus, these cells differed from the other two CD8+ subsets in that they were enriched for partly activated cells with a CD43hi, CD62Llo, CD69hi, and CD127lo phenotype (Fig. 1 B). As shown previously for total MP CD8+ cells (22), CD122hi MP CD8+ cells differed from naive CD44lo CD8+ cells in showing a slow tempo of proliferation, as measured by incorporation of the DNA precursor BrdU given for 3 or 7 d (Fig. 1 C). Note that proliferation of CD44lo cells is restricted to BrdU intermediate cells, which are recent thymic emigrants that incorporated a low level of BrdU in the thymus before export (22). Despite their similar surface markers, CD122hi MP CD8+ cells and naive CD8+ cells were clearly different with regard to Bcl-2 expression and IFN-γ production. Thus, relative to naive CD8+ cells, CD122hi MP CD8+ cells showed two- to three-fold higher levels of Bcl-2 (Fig. 1 D) and >10-fold higher production of IFN-γ upon in vitro stimulation (Fig. 1 E).
twofold less IFN-γ than the CD122hi subset of MP CD8+ cells (Fig. 1 E), suggestive of partial anergy.

Collectively, the data described above indicate that MP CD8+ cells can be divided into resting CD122lo cells and partly activated CD122hi cells. Nevertheless, some of the CD122hi cells seemed to be resting cells. The origin of these latter cells is discussed below.

Adoptive transfer of CD8+ cell subsets to normal B6 mice

The properties of the activated CD122lo subset of MP CD8+ cells suggested that these cells could be a short-lived population. To investigate this possibility, we purified CD8+ cell subsets by FACS. The purity of the CD8+ subsets after sorting was usually >99% for CD44hi cells and CD122hi CD44hi cells, and 88–90% for the CD122lo CD44hi subset (Fig. 2 A). The sorted CD8+ subsets were prepared from Thy1.1 congenic mice and adoptively transferred i.v. to normal young B6 mice (Thy1.2). The host mice were killed 7 d later to analyze LN and spleen cells by flow cytometry. The transferred CD44hi CD8+ cells accumulated preferentially in host LN, and most (>96%) of the cells maintained low levels of CD44, thus maintaining their naive phenotype (Fig. 2 B). The transferred CD122hi MP CD8+ cells homed equally well to the LN and spleen. Interestingly, the cells recovered from host LN were >95% CD122hi, whereas ~30% of the cells from the spleen were CD122hi cells, suggestive of CD122 down-regulation (see below). These latter CD122hi cells maintained their CD62Lhi phenotype (Fig. 2 C) and did not express the activation markers discussed above (not depicted).

The transferred CD44hi CD122lo CD8+ cells behaved differently. These cells homed preferentially to the spleen and a considerable fraction partly up-regulated CD122, both in the LN and spleen. As seen above for BrdU incorporation, the transferred MP CD122lo CD8+ cells proliferated considerably, yielding two- to threefold higher cell recoveries than for transferred CD122hi MP cells or CD44hi cells, especially in the spleen (Fig. 2, B and D). Like the initially injected cells, many of the recovered cells maintained a CD62Lhi phenotype (Fig. 2 C), contrasting with the CD122hi cells, suggestive of CD122 down-regulation (see below). These latter CD122hi cells maintained their CD62Lhi phenotype (Fig. 2 C) and did not express the activation markers discussed above (not depicted).

Therefore, the transfer experiments described above suggest that the CD122lo subset of MP CD8+ cells consists of a mixture of (a) partly activated cells and (b) resting MP cells that down-regulated their CD122 levels, perhaps in response to IL-15. In favor of this idea, brief (≈4 h) exposure of normal CD8+ cells to IL-15 in vitro at 37°C caused CD122hi cells to revert to CD122lo cells while remaining CD62Lhi (Fig. S2, available at http://www.jem.org/cgi/content/full/jem.20052495/DC1).

CD8+ cell subsets in γc−/− mice

As mentioned above, MP CD8+ cells in IL-15−/− and IL-15Rα−/− mice are nearly all CD122hi cells (13, 20, 21, and unpublished data). Interestingly, the few CD8+ cells present in γc−/− mice proved to be CD122hi cells (Fig. 3 A). These cells comprised a relatively homogeneous population with high intermediate expression of CD44 and low intermediate expression of CD122. Like MP CD8+ cells from

Figure 2. Phenotype of MP CD8+ cells on adoptive transfer. (A) Thy1.1-marked pooled LN cells from normal B6 mice were sorted by flow cytometry for CD44hi versus CD44lo CD122lo versus CD44hi CD122hi CD8+ T cells, resulting in the indicated purities after the sort. Sorted CD8+ cell subsets were then transferred i.v. at 1.5 × 106 cells/mouse to Thy1.2-marked WT mice, recovered 7 d later from pooled LN and spleen cells, and stained for FACS analysis. Transferred donor cells, identified as CD8+ Thy1.1+ cells, were examined for (B and C, left) CD44 versus CD122 and CD44/CD122 gates, CD44hi versus CD122hi cells in the same staining as shown. Numbers indicate percentages of cells in quadrants. (D) Total donor cell numbers (plus one standard deviation) were calculated for mice in B. The data are representative of at least two separate experiments.
IL-15−/− mice, γ−/− CD8+ cells were enriched for activation markers (Fig. 3 C and not depicted) and had a rapid turnover (Fig. 3 D).

These findings with γ−/− CD8+ cells suggested that the CD122lo MP CD8+ cells in normal mice may be independent of γ cytokines. If so, raising the level of γ cytokines would be expected to expand only the CD122hi and not the CD122lo subset of CD8+ cells. In line with this prediction, CD122lo cells comprised only a small proportion of MP CD8+ cells in mice expressing high levels of γ cytokines, namely IL-7 tg mice (Fig. 3 B; reference 14). In these mice, ~90% of MP CD8+ cells were CD122hi cells with a resting phenotype and slow turnover (Fig. 3 D and not depicted).

The enrichment of activated CD122lo MP CD8+ cells in IL-15−/− and γ−/− mice raised the possibility that these cells were not maintained by cytokines but by other stimuli, perhaps TCR interactions with MHC-I ligands. Indirect support for this possibility came from the finding that MHC-I−/− mice were virtually devoid of CD122lo MP CD8+ cells (Fig. 3 A). These mice contained MP CD8+ cells, presumably selected by MHC-Ib molecules (23), but these cells consisted almost entirely of CD122hi cells with a resting phenotype and slow turnover (Fig. 3 C and D).

**Transfer of CD122−/− MP CD8+ cells to MHC-I−/− hosts**

Collectively, the data described above suggest that γ cytokines are not essential for survival or background turnover of CD122lo CD44hi CD8+ cells. By 7 d after cell transfer, the donor CD4+ T cells had proliferated comparably well in WT and MHC-I−/− hosts as shown by CFSE dilution (Fig. 4 A). In marked contrast, the donor CD8+ T cells proliferated well in the WT mice but showed almost no division in MHC-I−/− recipients, resulting in 10-fold reduced cell recoveries from MHC-I−/− hosts compared with WT recipients (Fig. 4, A and B).

For the WT hosts, some of the proliferating donor CD8+ cells might be derived from contaminating naive cells, which can undergo MHC-I−dependent lymphopenia-induced proliferation. Therefore, we prepared purified (99%) CD44hi T cells from CD122−/− mice and adoptively transferred these cells to irradiated WT versus MHC-I−/− mice. Again, 7 d after adoptive transfer, the donor CD4+ T cells showed comparable division profiles in both groups as shown by CFSE dilution, whereas the donor CD8+ T cells proliferated much less in MHC-I−/− recipients than in WT hosts (Fig. 4 C). This difference was also seen when the transferred cells were enriched for CD44hi T cells (~90%) by magnetic cell sorting (Fig. 4 D). In contrast to these experiments using T cells from CD122−/− mice, when sorted CD44hi T cells from normal B6 WT mice were used as donor cells, the donor CD8+ T cells proliferated equally well in both hosts (Fig. 4 E), demonstrating that CD122hi MP CD8+ cells expand in an MHC-I−independent fashion (24).

Thus, these experiments show that proliferation of CD122−/− CD44hi CD8+ cells is heavily dependent on contact with MHC-I molecules.

**Transfer of normal MP CD8+ cells to MHC-I−/− hosts**

Despite their similar phenotype, MP CD8+ cells from CD122−/− mice might not necessarily be equivalent to the CD122lo subset of MP CD8+ cells in normal mice. Hence, it remained important to test the MHC-I dependency of normal CD122lo MP CD8+ cells. Therefore, the experiments described above were repeated with purified MP CD8+ cells prepared from normal B6 mice. Rather than injecting purified CD122lo MP CD8+ cells, which are difficult to prepare in more than minimal numbers from normal mice (Fig. 2), we transferred purified normal B6 MP CD8+ cells
(i.e., a mixture of CD122lo CD44hi and CD122hi CD44hi cells) to irradiated WT versus MHC-I–/– hosts (Fig. 5 A). When the injected cells were recovered 1 wk later, a high proportion of the donor CD44hi cells (90%) were CD122hi, presumably reflecting homeostatic expansion of these latter cells in response to the raised levels of γc cytokines in the lymphopenic environment of the irradiated hosts (14). The few donor CD122lo cells in the WT hosts were enriched for cells with an activated CD62Llo CD43hi phenotype, consistent with chronic activation to MHC-I ligands (Fig. 5 B). Significantly, cells with this activated phenotype were quite rare in the MHC-I–/– hosts. In these hosts, the donor MP CD8+ cells were enriched for CD122hi cells and the minor subset of donor CD122lo cells had a resting CD62Lhi CD43lo phenotype, consistent with these cells being revertants from CD122hi cells (Fig. 5 B). In terms of cell numbers, total numbers of CD62Llo and CD43hi subsets of donor CD44hi CD8+ T cells were greatly reduced in MHC-I–/– hosts; i.e., by fivefold and eightfold, respectively (Fig. 5 C). In contrast, total numbers of donor CD122hi cells in WT and MHC-I–/– hosts were comparable (Fig. 5 D).

The above findings indicated that as for CD8+ cells from CD122–/– mice, the subset of activated CD122lo MP CD8+ cells in normal mice disappeared rapidly after transfer to MHC-I–/– hosts.

Transfer to nonirradiated MHC-I–/– hosts
A complicating feature of the experiments described above is that irradiation of the hosts to prevent rejection caused the donor cells to undergo lymphopenia-induced homeostatic proliferation. Hence, it was important to have comparable information on cell survival in nonirradiated MHC-I–/– hosts. Here, the main problem is rejection by residual host CD8+ cells. Because rejection by MHC-I–/– hosts is directed to MHC-I molecules on the donor cells, we avoided the problem of rejection by preparing donor cells that lacked MHC-I; i.e., by reconstituting heavily irradiated normal B6 WT mice...
with BM cells from MHC-I<sup>-/-</sup> mice (25). The MHC-I<sup>-/-</sup> CD4<sup>+</sup> and CD8<sup>+</sup> cells generated in these MHC-I<sup>-/-</sup>→WT chimeras closely resembled the cells generated in control WT→WT chimeras (Fig. 6 A). In contrast, CD4<sup>+</sup> cells, but very few CD8<sup>+</sup> cells, were generated in reciprocal WT→MHC-I<sup>-/-</sup> and MHC-I<sup>-/-</sup>→MHC-I<sup>-/-</sup> chimeras.

For adoptive transfer experiments, we used purified donor Thy1-marked T cells from MHC-I<sup>-/-</sup> (Thy1.1)→WT (Thy1.2) chimeras. These MHC-I<sup>-/-</sup> T cells, a mixture of CD4<sup>+</sup> and CD8<sup>+</sup> cells, were then transferred to WT versus MHC-I<sup>-/-</sup> mice (both Thy1.2). To avoid rejection by NK cells, the hosts had been pretreated with anti-NK1.1 mAb. With transfer to WT hosts, the phenotype of the donor MP CD8<sup>+</sup> cells remained constant and there was no decline in the proportion of CD122<sup>hi</sup> cells (Fig. 6 B). With transfer to MHC-I<sup>-/-</sup> hosts, in contrast, the proportion of CD122<sup>hi</sup> MP CD8<sup>+</sup> cells declined abruptly between days 1 and 14 after transfer, leading to a reciprocal relative increase in CD122<sup>lo</sup> cells (Fig. 6 C). This decrease in CD122<sup>hi</sup> MP CD8<sup>+</sup> donor cells was also evident from total cell recoveries after adoptive transfer. Thus, for the donor MP CD8<sup>+</sup> cells, the total recovery of CD122<sup>hi</sup> cells was the same in WT and MHC-I<sup>-/-</sup> hosts on day 14, whereas the recovery of CD122<sup>lo</sup> cells was four-fold lower in MHC-I<sup>-/-</sup> hosts than in WT hosts (Fig. 6 D). There was no change in CD4<sup>+</sup> cells (not depicted).

Collectively, the above three sets of experiments with MHC-I<sup>-/-</sup> hosts indicate that the subset of MP CD8<sup>+</sup> cells with an activated CD122<sup>hi</sup> phenotype is MHC-I dependent and is presumably engaged in chronic TCR responses to MHC-I ligands.

**DISCUSSION**

As mentioned earlier, T cells exhibiting the properties and features of antigen-specific memory cells arise early in life and become a dominant population in old age. The prevailing view is that these naturally occurring MP T cells are the progeny of naive precursors responding to various environmental antigens. However, some MP T cells may arise through contact with self-antigens rather than foreign antigens. This possibility is supported by the finding that naive T cells undergo “homeostatic” proliferation and differentiation into typical MP cells during T lymphopenia, e.g., in the normal neonatal period or when adult mice are depleted of T cells (26–28). This proliferative response is directed largely to self-antigens and is stimulated by the raised levels of γc cytokines, especially IL-7, that occur when total T cell levels are low. What proportion of MP cells are driven by self-rather than foreign antigens in normal mice is unclear. Nevertheless, it is notable that MP cells are readily detectable in germ-free mice and even in “antigen-free” mice fed an amino acid diet (29, 30, 31 and unpublished data). Thus, most MP cells may be the progeny of self-reactive cells. Characterizing the features of MP cells is therefore important.

For MP CD8<sup>+</sup> cells, we show here that these cells comprise two broad subsets: (a) a major population of resting CD122<sup>lo</sup> CD44<sup>hi</sup> cells and (b) a minor subset of partly activated...
CD122lo MP CD8+ cells. The CD122hi subset closely resembled naive CD44hi CD8+ cells by several surface markers but was distinct in two respects. First, confirming previous findings (13, 22), background proliferation of CD122hi cells was slow but significant; in contrast, naive CD44hi CD8+ cells rarely divided. Second, unlike naive CD8+ cells, CD122lo MP CD8+ cells proved to be MHC-I independent. Thus, CD122lo cells expanded in MHC-I−/− hosts, whereas naive cells gradually disappeared (Fig. 6 and not depicted).

Unlike CD122lo cells, the CD122hi component of MP CD8+ cells had a rapid turnover and was enriched in cells with activated phenotype. These cells were spread throughout the secondary lymphoid tissues, including peripheral LNs, suggesting the cells were activated by a pervasive rather than a local stimulus. As in IL-15−/− mice, CD122hi MP CD8+ cells were enriched in γδ+ mice, indicating a lack of dependence on γδ cytokines. Although reliance on other cytokines cannot be excluded, the cells are probably maintained largely by TCR stimuli. This follows from the finding that CD122hi MP CD8+ cells disappeared rapidly and ceased to proliferate after transfer to MHC-I−/− hosts. It should be noted that a significant proportion of CD122hi MP CD8+ cells (30–50%) were MHC-I independent and had a resting phenotype. Based on the effects of exposing cells to IL-15 in vitro, resting CD122lo MP CD8+ cells are probably “revertants” of CD122hi cells responding to IL-15.

A key issue is whether the activated CD122lo subset of MP cells can be equated with a subset of antigen-specific memory cells. In the case of CD122hi MP CD8+ cells, these cells closely resemble typical long-lived, antigen-specific “central” memory cells (4) by multiple parameters, including surface markers, turnover, IL-15 dependency, and lack of dependence on MHC-I. It might then follow that CD122hi MP CD8+ cells are the counterpart of “effector” memory cells. This is unlikely for several reasons (32–34). First, despite their CD62L+ phenotype, effector memory cells do not display activation markers. Second, whereas CD122lo MP CD8+ cells have a rapid turnover, effector memory CD8+ cells have a slow turnover. Third, in terms of IFN-γ production, CD122lo MP cells are anergic, whereas effector memory cells respond as effectively as central memory cells. Fourth, unlike CD122hi MP cells, effector memory cells are CD122lo cells. Fifth, unlike effector memory cells, CD122lo MP CD8+ cells are proportionally as frequent in LNs as in the spleen. Hence, activated CD122lo MP CD8+ cells cannot be equated with either effector or central memory cells.

Although clearly different from classical memory cells, CD122lo MP CD8+ cells closely resemble a population of antigen-specific memory CD8+ cells that develops during chronic viral infections in mice (35–37). These cells have low levels of CD122, IL-7Rα, CD62L, and Bcl-2, express higher levels of CD43 as well as CD69, and display partial anergy in terms of antigen responsiveness. Significantly, CD8+ cells in chronic infections rapidly disappear when deprived of contact with specific antigen, suggesting that the cells are maintained largely by chronic TCR contact with persisting antigen. Interestingly, CD8+ cells found in HIV-infected individuals are enriched in cells carrying memory markers as well as low levels of IL-7Rα and CD62L, show a higher in vivo proliferation rate, and are more susceptible to apoptosis and partially anergic in vitro (38). Thus, the memory CD8+ cells generated during chronic infection have much in common with the naturally occurring population of CD122lo MP CD8+ cells described here.

The MHC-I ligands recognized by CD122lo MP CD8+ cells are unclear. The possibility we favor is that these cells are reacting to the same self-ligands that drive homeostatic proliferation in lymphopenic hosts. In favor of this hypothesis, we found similar Vβ TCR usage within CD122lo MP CD8+ cells and polyclonal CD8+ T cells undergoing homeostatic expansion (Fig. S4, A and B, available at http://www.jem.org/cgi/content/full/jem.20052495/DC1).

In normal hosts, proliferation to self MHC-I ligands might be limited to a small subset of naive CD8+ cells with “above-average” affinity for self. If so, what is the fate of the responding CD122lo cells? One possibility is that CD122lo cells resemble typical effector cells in having a short lifespan; the cells proliferate in brief but most of the cells then die. However, CD122lo MP CD8+ cells differ from typical effector cells in that they are CD25lo and display partial anergy, at least for IFN-γ synthesis. More importantly, the transfer studies showed that at a population level, purified CD122lo cells did not die but survived quite well in WT hosts, with some of the cells differentiating into CD122hi cells. Hence, the data favor a model in which contact with self-MHC-I ligands drives CD8+ cells to proliferate and then differentiate from semi-activated CD122lo cells into resting CD122hi cells. To maintain homeostasis, cell expansion here is presumably balanced by an equivalent rate of cell death, but how such immunoregulation is controlled is unclear. It is also a mystery that differentiation into CD122lo cells causes CD8+ cells to lose their MHC-I dependency. Future studies will be required to resolve these issues.

MATERIALS AND METHODS

Mice. C57BL/6 (B6), Thy1.1 (B6.PL), CD122-deficient (CD122−/−), and γδ-deficient (γδ−/−) mice, all on a B6 background, were purchased from The Jackson Laboratory. Dβ c–/– (B6.Dbc–/–), B6.B6–/– (B6.B6–/–), B6.B6–/– (B6.B6–/–), and B6.B6–/– (B6.B6–/–) mice were purchased from The Jackson Laboratory. MHC-I−/− (B6.H-2−/−) mice, all on a B6 background (16) were provided by R. Ahmed (Emory University, Atlanta, GA) and maintained in our animal facility. IL-15−/− (B6.I-15−/−) mice (13) and IL-7−/− mice (14) were maintained in our animal facility. All mice were housed under specific pathogen-free conditions at The Scripps Research Institute and used at 3–6 mo of age. Experiments involving the use of animals were approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute.

Flow cytometry and cell sorting. Cell suspensions of spleen or pooled (inguinal, axillary, cervical, and mesenteric) LNs were prepared according to standard protocols and stained for FACS analysis or sorting using PBS containing 1% FCS and 2 mM EDTA with the following mAbs (from BD Biosciences unless otherwise stated): PerCP-Cy5.5–conjugated anti-CD3 (145-2C11); Alexa Fluor 405–conjugated anti-CD4 (RM4-5; Caltag Laboratories); PerCP-Cy5.5– or APC-Cy7–conjugated anti-CD8α (53-6.7); PE-conjugated anti-CD8β (H57-16.2); FITC–conjugated anti-CD25 (PC61.5); PE-conjugated anti-CD43 (B11); APC–conjugated anti-CD44 (IM7; eBioscience); FITC– or PE–conjugated anti-CD62L (MEL-14);
FITC-conjugated anti-CD69 (H1.2F3); APC-conjugated anti-CD90.1 (HIS51, eBioscience); FITC– or PE-conjugated anti-CD122 (TM-B1 or alternatively 5H4); PE-conjugated anti-CD127 (SB/14); and PE-conjugated anti-CD132 (4G3). For staining of the intracellular markers Bel-2 and BrdU, the Bel-2 reagent set containing FITC-conjugated anti-Bel-2 mAb (3F11) or FITC-conjugated isotype-matched control mAb and the FITC BrdU flow kit (both from BD Biosciences) were used, respectively, according to standard protocols (22). In brief, cells were stained for cell surface markers, fixed using 2% paraformaldehyde, and permeabilized using saponin before intracellular staining. Flow cytometry samples were analyzed using a BD LSR II digital flow cytometer. For determining Vβ TCR usage, the Vβ TCR Screening Panel from BD Biosciences was used according to the manufacturer’s recommendation.

Measurement of cell turnover in vivo. Proliferation of cells in vivo was measured using dilution of CFSE-labeled cells (13) or incorporation of BrdU (0.8 mg/ml) given in the drinking water (22). CFSE staining was performed as follows: cells were resuspended in PBS containing 1% FCS at 10–20 × 10^6 cells/ml and stained with 1 μl of 5 mM Vybrant CFDA SE Cell Tracker dye (Invitrogen) per milliliter of cell suspension for 10 min at 37°C, and then washed twice with ice-cold PBS containing 1% FCS. BrdU staining was performed as described above.

In vitro stimulation and IFN-γ measurement by ELISA. Spleen cells from normal B6 mice were sorted by FACS for CD44^hi, CD122^lo, CD44^lo CD122^lo, CD44^lo CD122^hi, and CD44^hi CD122^hi CD8^+ T cell subsets were then transferred i.v. at 1.5 × 10^6 Thy1.2-marked WT or MHC-I–/– mice. Seven days after transfer, mice were killed, and spleen cells were depleted using mAbs against heat-stable antigen (J11d), Thy1.2 (J1.10), and MHC-II (28-16-8s) plus complement (40). Purity was routinely >99% for CD44^lo, 88–90% for CD44^hi, 99% for CD44^lo, and 99% for CD44^hi CD122^hi. CD8^+ T cell subsets were then transferred in triplicates at 7 × 10^5 cells/well to sublethally irradiated (750 cGy) Thy1.1-marked WT or MHC-I–/– mice. Seven days after transfer, mice were killed, and spleen cells were depleted using mAbs against heat-stable antigen (J11d), Thy1.1 (T24), CD4 (RL172), CD8 (3.168), and MHC-II (28-16-8s) plus complement. Contamination of purified BM cells with mature B, T, or MHC-II–expressing cells was <2%. Recipient Thy1.2-marked WT or MHC-I–/– mice were irradiated with 1,300 cGy before i.v. injection of 5 × 10^5 purified BM cells. Recipients were given antibiotics in their drinking water for the first 3–4 wk and left for 3–4 mo to allow for de novo T cell generation. Donor BM-derived T cells were then collected by Thy1.1 and purified using mAbs against heat-stable antigen (J11d), Thy1.2 (J1.10), and MHC-II (28-16-8s) plus complement as described above. 2–3 × 10^6 Thy1.1 T cells were injected i.v. into Thy1.2 WT or MHC-I–/– mice, which had been depleted of NK cells using anti-NK.1.1 ascites fluid (PK136) on days −3 and −1 before adoptive transfer and every other day thereafter.

Online supplemental material. Fig. S1 shows expression of CD25, CD122, γc, CD4, CD44, CD44, CD62L, CD69, and CD127 on antigen-specific naive, effector, and memory CD8^+ T cells during a response to lymphocytic choriomeningitis virus. Fig. S2 shows that, in vitro, IL-15 leads to down-regulation of CD122 on MP CD8^+ T cells without affecting their CD62L levels. Fig. S3 shows that CD8^+ MP cells from CD122^−/− mice are comparable to the CD122^+ subset of MP CD8^+ T cells from WT mice. Fig. S4 shows Vβ TCR usage within CD8^+ T cell subsets and within CD8^+ T cells undergoing homeostatic expansion. Figs. S1–S4 are available at http://www.jem.org/cgi/content/full/jem.20052495/DC1.

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