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 display 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.

Depending on their stage of differentiation, mature T cells require contact with peptide/MHC complexes and/or cytokines, most notably the common γ chain (γc) cytokines IL-7 and IL-15 (1–4). Naive CD4⁺ T cells show low expression of CD44, and these CD44lo cells are controlled by IL-7 and possibly MHC-II molecules, although the importance of the latter is controversial (5–7). The factors controlling naturally occurring memory phenotype (MP) CD44hi CD4⁺ are not well characterized, although antigen–specific memory CD4⁺ cells are known to be sustained by IL-7 and, to a lesser extent, IL-15 (8–10). For CD8⁺ cells, naive CD44lo cells are maintained by IL-7 and MHC-I molecules (5, 11, 12), whereas MP and antigen–specific memory cells are controlled by IL-15 and IL-7 and do not require contact with MHC-I complexes (13–16).

For naive T cells, TCR contact with foreign antigen causes CD44lo cells to convert to CD44hi cells and show transient up-regulation of several surface markers, including IL-2Rα (CD25), CD69, and CD43 (1–4). Many of the responding cells also show decreased levels of CD62L (L-selectin) and IL-7Rα (CD127). Interestingly, naive T cells normally maintain a resting (CD25lo, CD69lo, CD43lo, CD127hi) phenotype despite continuous TCR–MHC interaction delivering only a weak signal, adequate for maintaining cell viability but insufficient for overt stimulation and subsequent proliferation. Except for their CD44hi phenotype, MP CD8⁺ cells show a similar pattern of cell surface markers as naive CD8⁺ cells, with the exception that ~60–70% of MP CD8⁺ cells display high levels of the IL-2/IL-15RBβ chain (CD122). This receptor chain is crucially important for controlling sensitivity to IL-15 (13, 17). Thus, CD122hi MP CD8⁺ cells are overrepresented in IL-15 transgenic (tg) mice (18, 19). Conversely, IL-15–/– (13, 20) and IL-15Rα–/– (21) mice show a marked and selective reduction of CD122hi MP CD8⁺ cells. This subset of MP CD8⁺ cells is highly dependent on IL-15 for IFN-induced bystander proliferation as well as for normal...
“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 MP CD4+ and MP CD8+ cells, expression of the IL-2/IL-15Rα (CD122) is considerably higher on MP CD8+ than MP CD4+ cells, as described previously (13, 17). Thus, plotting CD44 against CD122 divides CD8+ cells into three subsets: CD122lo CD44lo (naive), CD122lo CD44hi CD8+ MP, and CD122hi CD44hi MP cells. The majority of MP CD8+ cells (∼60–70%) carries high levels of CD122 (Fig. 1 B). As shown previously for total MP CD8+ cells (22), CD122hi MP CD8+ cells differed from naive CD44lo CD8+ cells for several markers; thus, both subsets consisted almost exclusively of resting T cells characterized by a CD25lo, CD43lo, CD62Llo, CD69lo, and CD127hi 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 significant but 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 BrdUintermediate 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 CD122lo 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, CD69lo, CD127hi phenotype (Fig. 1 B), thereby resembling TCR-activated T cells (Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20052495/DC1). CD122lo MP cells also showed low levels of Bcl-2 (Fig. 1 D). However, unlike overtly activated T cells stimulated by strong TCR ligation, the levels of CD25 (IL-2Rα) and CD132 (γc) on CD122lo MP CD8+ cells were not elevated (relative to resting T cells). In line with their expression of activation markers, CD122lo MP CD8+ cells divided two to three times faster than the CD122hi MP CD8+ subset (Fig. 1 C). Also, CD122lo CD44hi CD8+ cells produced...
twofold less IFN-γ than the CD122lo 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 CD122hi subset of MP CD8+ cells and partly activated CD122lo cells. Nevertheless, some of the CD122lo 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 CD44lo 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 CD44lo 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 CD122lo cells, suggestive of CD122 down-regulation (see below). These latter CD122lo 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 CD122lo cells derived from the transferred MP CD122hi CD8+ cells that were purely CD62Lhi. Upon CD122 up-regulation, however, the transferred CD44hi CD122lo CD8+ cells became predominantly CD62Lhi (Fig. 2 C).

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 CD122lo cells to revert to CD122hi 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-15Ra–/– mice are nearly all CD122hi (13, 20, 21, and unpublished data). Interestingly, the few CD8+ cells present in γc–/– mice proved to be CD122lo 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
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 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 or CD44hi CD8+ cells. The data show staining of gated CD3−, CD8+, CD69, and CD43 (top) or CD3, CD8, CD69, and CD43 (bottom) and analyzed by flow cytometry. The data show staining of gated CD3−, CD8+ cells. Therefore, the experiments described above were repeated with purified MP CD8+ cells prepared from normal B6 mice. Rather than injecting purified CD122hi 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 being killed and intracellular BrdU staining.
(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 \( \gamma_c \) 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+ 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 CD8+ T cells were calculated and are shown for (C) CD122hi CD62Lhi and CD122hi CD43hi cells, and (D) CD122hi CD62Lhi CD43hi cells (resting CD122hi cells) recovered from the spleens and pooled LNs of WT (filled bars) versus MHC-I–/– (open bars) hosts. Numbers above the bars indicate the ratios of total cells from WT versus MHC-I–/– hosts. The data are representative of at least two separate experiments.

Figure 4. Fate of CD122–/– T cells transferred to irradiated MHC-I–/– hosts. Irradiated WT versus MHC-I–/– hosts were injected i.v. with (A–D) CFSE-labeled T cells (2–3 \( \times 10^6 \) cells/mouse) from CD122–/– mice or (E) purified and CFSE-labeled CD44hi T cells (2–3 \( \times 10^6 \) cells/mouse) from normal B6 WT mice. The cells transferred were (A and B) unseparated CD122–/– T cells prepared by complement-mediated killing with mAbs, (C) FACS-sorted CD44hi CD122–/– T cells, (D) CD8+ CD122–/– cells enriched for CD44hi cells by magnetic bead separation, or (E) FACS-sorted CD44hi T cells from normal B6 WT mice. The host mice received (A, B, and E) a single dose of 750 cGy or (C and D) two doses of 600 cGy 2 wk apart. CFSE dilution of the cells recovered from host spleen was examined 7 d later. Histograms show CFSEhi CD4+ or CD8+ donor cells, respectively. Numbers indicate percentages of cells in quadrants. (B) Total cell numbers (plus one standard deviation) of donor CD4+ and CD8+ T cells recovered from the spleens and pooled LNs of WT (filled bars) versus MHC-I–/– (open bars) hosts treated as in A (two mice/group). Numbers above the bars indicate the ratios of total cells from WT versus MHC-I–/– hosts. p-values of WT compared with MHC-I–/– cell numbers were calculated using a paired \( t \) test. *, not significant (\( P = 0.4 \)); **, significant (\( P = 0.02 \)). The data are representative of at least two different experiments.

Figure 5. Fate of normal B6 MP T cells transferred to irradiated MHC-I–/– hosts. Purified CD44hi T cells from Thy1.1-marked normal B6 WT mice were transferred i.v. at 1.5 \( \times 10^6 \) cells/mouse to irradiated (750 cGy) Thy1.2-marked WT or MHC-I–/– mice. 7 d after transfer, mice were killed and analyzed by flow cytometry for donor (Thy1.1+ CD8+ and CD4+ cells) (A), and for CD62L and CD43 expression on donor CD122lo CD44hi CD8+ cells (B, left two columns) versus CD122hi CD44hi CD8+ cells (B, right two columns). Total numbers of donor CD44hi CD8+ T cells were calculated and are shown for (C) CD122hi CD62Lhi and CD122hi CD43hi cells, and (D) CD122hi CD62Lhi CD43hi cells (resting CD122hi cells) recovered from the spleens and pooled LNs of WT (filled bars) versus MHC-I–/– (open bars) hosts. Numbers above the bars indicate the ratios of total cells from WT versus MHC-I–/– hosts. The data are representative of at least two separate experiments.

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−/− mice (25). The MHC-I−/− CD4+ and CD8+ cells generated in these MHC-I−/−→WT chimeras closely resembled the cells generated in control WT→WT chimeras (Fig. 6 A). In contrast, CD4+ cells, but very few CD8+ cells, were generated in reciprocal WT→MHC-I−/− and MHC-I−/−→MHC-I−/− chimeras.

For adoptive transfer experiments, we used purified donor Thy1.1-marked T cells from MHC-I−/− (Thy1.1)→WT (Thy1.2) chimeras. These MHC-I−/− T cells, a mixture of CD4+ and CD8+ cells, were then transferred to WT versus MHC-I−/− 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+ cells remained constant and there was no decline in the proportion of CD122hi cells (Fig. 6 B). With transfer to MHC-I−/− hosts, in contrast, the proportion of CD122hi MP CD8+ cells declined abruptly between days 1 and 14 after transfer, leading to a reciprocal relative increase in CD122lo cells (Fig. 6 C). This decrease in CD122hi MP CD8+ donor cells was also evident from total cell recoveries after adoptive transfer. Thus, for the donor MP CD8+ cells, the total recovery of CD122hi cells was the same in WT and MHC-I−/− hosts on day 14, whereas the recovery of CD122lo cells was four-fold lower in MHC-I−/− hosts than in WT hosts (Fig. 6 D). There was no change in CD4+ cells (not depicted).

Collectively, the above three sets of experiments with MHC-I−/− hosts indicate that the subset of MP CD8+ cells with an activated CD122hi 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+ cells, we show here that these cells comprise two broad subsets: (a) a major population of resting CD122hi CD44hi cells and (b) a minor subset of partly activated...
CD122hi CD44hi cells. The CD122lo 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 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 CD122hi cells, the CD122lo 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 LN, suggesting the cells were activated by a pervasive rather than a local stimulus. As in IL-15−/− mice, CD122lo MP CD8+ cells were enriched in γδ T cells, 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 CD122hi 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 CD62Llo phenotype, effector memory cells do not display activation markers. Second, whereas CD122hi MP CD8+ cells have a rapid turnover, effector memory CD8+ cells have a slow turnover. Third, in terms of IFN-γ production, CD122hi MP cells are anergic, whereas effector memory cells respond as effectively as central memory cells. Fourth, unlike CD122hi MP cells, effector memory cells are CD122hi cells. Fifth, unlike effector memory cells, CD122lo MP CD8+ cells are proportionally as frequent in LN 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 CD122hi MP CD8+ cells described here.

The MHC-I ligands recognized by CD122hi 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 CD122hi 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 CD122hi 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 γc-deficient (γc−/−) mice, all on a B6 background, were purchased from The Jackson Laboratory. Dβh−/− Kβ−/− β2M−/− MHC-I−/− mice on a B6 background (16) were provided by R. Ahmed (Emory University, Atlanta, GA) and maintained in our animal facility. IL-15−/− mice (15–24 mo of age) 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) LN were prepared according to standard protocols and stained for FACScan 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 (H57-17.2); FITC–conjugated anti-CD25 (PC61.5); PE–conjugated anti-CD43 (1B11); APC–conjugated anti-CD44 (IM7; eBioscience); FITC− or PE–conjugated anti-CD62L (MEL-14);
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 ~20–20 × 10^6 cells/ml and stained with 1 μl of 5 nM 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^, CD44^lo^, and CD44^lo^ CD122^lo^ cells. These purified CD8^+^ cells were then seeded in a V-bottom 96-well plate in triplicates at 7 × 10^4 cells/well and stimulated with 50 ng/ml PMA and 500 ng/ml ionomycin for 4 h under normal culture conditions (37°C, 7% CO₂, humidified atmosphere). Supernatants were then collected and assayed for IFN-γ by a sandwich ELISA according to standard protocols, as described previously (39). In brief, culture supernatants were diluted serially in PBS and added to flat-bottom 96-well plates coated with a purified mAb specific for IFN-γ (XMG1.2; eBioscience). Known concentrations of recombinant murine IFN-γ were used as standards. After incubation for 2 h at 37°C, the plates were washed and biotinylated mAb (R4-6A2; eBioscience) was added for 1 h at 37°C. After washing again, streptavidin-conjugated horseradish peroxidase was added for 1 h at room temperature. The samples were then developed using the substrate O-phenylenediamine (Sigma-Aldrich) and, after stopping the reaction with 2 N H₂SO₄, analyzed with an ELISA reader (Spectramax Plus 384; Molecular Devices).

Adoptive transfer of purified CD8^+^ T cell subsets. Pooled LN cells from Thy1.1-marked WT or IL-7 tg mice were surface-stained for CD122, CD8β, and CD44 and sorted using a Becton Dickinson FACS Aria. Purities of FACS-sorted CD8^+^ T cell subsets were >99% for CD44^lo^, 88–90% for CD44^hi^ CD122^lo^, and >99% for CD44^hi^ CD122^hi^ cells. CD8^+^ T cell subsets were then transferred i.v. at 1.5 × 10^6 cells/mouse to Thy1.1-marked WT mice and left for 7 d before mice were killed. IL-7 tg mice were used in some experiments as they contained higher numbers of total spleen and LN cells enriched for CD44^lo^ CD8^+^ T cells (14), which, divided into CD122^lo^ and CD122^hi^ cells, were indistinguishable from the corresponding subsets in WT mice by all parameters tested (Fig. 3 and unpublished data). For experiments involving whole T cells, pooled LN cells from CD122^lo^ mice were subjected to complement-mediated killing by treating cell suspension with anti–heat-stable antigen mAb (J11d) and anti–MHC-II mAb (28–16–8) plus complement (4©). Purity was routinely >95%. Cells were then labeled with CFSE (see above) and injected i.v. at 2–3 × 10^6 T cells per recipient. Recipients were irradiated with 750 cGy 6–8 h before adoptive transfer. For CD122^lo^ CD8^+^ T cell subsets, LN cells from CD122^lo^ mice were prepared as described above and subsequently sorted by FACS. The purity of FACS-sorted CD8^+^ CD44^lo^ cells was ~99%. Alternatively, CD122^lo^ CD8^+^ cells were enriched for CD44^lo^ cells by negative depletion of LN cells using MACS CD8^+^ T cell isolation kit (Miltenyi Biotec) in combination with biotinylated anti-CD62L mAb (MEL-14). In brief, pooled LN cells from CD122^lo^-marked mice were incubated with biotinylated mAbs against CD4 (L3T4), CD45R (B220), CD49b (DX5), CD11b (Mac-1), Ter-119, and CD62L, followed by incubation with anti-biotin MicroBeads and negative depletion of labeled cells. Typical purities of sorted cells were ~90%. CD122^lo^ CD44^lo^ CD8^+^ cells were then CFSE labeled and adoptively transferred i.v. at 2–3 × 10^6 cells per host. Recipient mice underwent split-dose irradiation of 600 cGy 2 wk before plus 600 cGy 6–8 h before adoptive transfer.

Mixed BM radiation chimeras. BM cells were obtained from Thy1.1-marked WT or MHC-I−/− mice, and mature B, T, and MHC-II-expressing cells were depleted using mAbs against heat-stable antigen (J11d), Thy1.1 (T24), CD4 (RL172), CD8 (3.168), and MHC-II (28–16–8) plus complement. Contamination of purified BM cells with mature B, T, and MHC-II-expressing cells was <2%. Recipient Thy1.2-marked WT or MHC-I−/− mice were irradiated at 1,300 cGy before i.v. injection of 5–10 × 10^6 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 identified by Thy1.1 and purified using mAbs against heat-stable antigen (J11d), Thy1.2 (J1,10), and MHC-II (28–16–8) 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-NK1.1 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, γ, CD43, 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^+^ cells without affecting their CD62L level. Fig. S3 shows that CD8^+^ MP cells from CD122^lo^-marked mice are comparable to the CD122^lo^ subset of MP CD8^+^ 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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