with the observation that thymocyte proliferation is not affected in mice expressing dominant negative MEK1 (*9*). Ablation of p44 MAPK appeared not to affect the long-term total MAPK activity in MEFs or thymocytes. This result suggests that both isoforms might compete with each other for the upstream MEK activator. These findings indicate that there may be a physiological distinction between p42 and p44 MAPK isoforms.

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Persistence of Memory CD8 T Cells in MHC Class I–Deficient Mice

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An understanding of how T cell memory is maintained is crucial for the rational design of vaccines. Memory T cells were shown to persist indefinitely in major histocompatibility complex (MHC) class I–deficient mice and retained the ability to make rapid cytokine responses upon reencounter with antigen. In addition, memory CD8 T cells, unlike naïve cells, divided without MHC–T cell receptor interactions. This "homeostatic" proliferation is likely to be important in maintaining memory T cell numbers in the periphery. Thus, after naïve CD8 T cells differentiate into memory cells, they evolve an MHC class I–independent "life-style" and do not require further stimulation with specific or cross-reactive antigen for their maintenance.

Immunological memory is the ability of the immune system to respond with greater vigor upon reencounter with the same pathogen. Many currently used vaccines and most natural infections induce long-term T cell memory as assessed by rapid anamnestic responses (*1–3*). These accelerated recall responses are due to increased numbers of antigen-specific T cells and to qualitative changes in memory T cells that allow them to develop into effector cells more rapidly than naïve T cells (*2–4*). There has been considerable interest in determining whether continued presentation of specific or cross-reactive antigens by MHC molecules is necessary for maintenance of memory T cells and for retaining their "response ready" mode (*1–6*). We have addressed this issue by analyzing the survival, proliferation, and functional characteristics of memory CD8 T cells under conditions of MHC class I deficiency.

Adult mice resolve an acute lymphocytic choriomeningitis virus (LCMV) infection within 2 weeks and then exhibit long-term CD8 T cell memory (*2*, *3*). To determine the requirement of MHC class I molecules in maintaining CD8 memory, we obtained fluorescence-activated cell sorter (FACS)–purified CD8 T cells (>99% pure) from Thy1.1$^+$ LCMV immune mice and transferred the cells into either MHC class I–positive ($\beta_2M^{+/+}$) or MHC class I–deficient ($\beta_2M^{-/-}$) congeneic Thy1.2$^+$ mice. Before adoptive transfer, the FACS-purified CD8 T cells were tested for the presence of virus and found to be free of any detectable viral material (*7*). The transferred CD8 T cells initially expanded in the irradiated recipient mice, reaching a plateau at around day 20, and were then maintained indefinitely in both $\beta_2M^{+/+}$ and $\beta_2M^{-/-}$ mice (Fig. 1A). Although the initial expansion of CD8 T cells was less in $\beta_2M^{-/-}$ mice, by day 22 the numbers were similar in both groups of mice. The LCMV-specific memory CD8 T cells were CD44^{hi}, Ly6C^{hi}, CD25^{lo}, and CD69^{lo} before transfer, and this phenotype was retained in MHC class I–deficient mice. The total Thy1.1$^+$ CD8 T cell population initially consisted of equal numbers of CD44^{lo} (naïve) and CD44^{hi} (memory) cells. However, by day 50 nearly all of the donor Thy1.1$^+$ CD8 T cells were CD44^{hi}, suggesting preferential survival of memory cells in $\beta_2M^{-/-}$ mice (*8*).

Peripheral T cells undergo homeostatic proliferation under lymphopenic conditions to reconstitute the empty immune system (*5*). When lymphoid homeostasis is reached, naïve cells cease to proliferate, whereas memory cells continue to divide, although at a much slower rate, in order to maintain the pool of memory cells (*9*). We sought to determine whether memory CD8 cells persisting in MHC class I–deficient mice at relatively constant numbers after reaching lymphoid homeostasis (between day 22 and day 310) (Fig. 1A) were undergoing proliferation or surviving without any cell division. The turnover rate of LCMV-specific memory CD8 T cells (identified by staining with MHC class I tetramers) was similar in $\beta_2M^{+/+}$ and $\beta_2M^{-/-}$ mice (Fig. 1B). Likewise, the total population of donor Thy1.1$^+$ CD8 T cells, which consist predominantly of non-LCMV–specific CD44^{hi} CD8 T cells, proliferated equally well in +/+ and $\beta_2M^{-/-}$ mice. Thus, not only do memory CD8 T cells persist in $\beta_2M^{-/-}$ mice, but their homeostatic proliferation is not compromised in a heavily MHC class I–deficient environment.

In the experiments described above the donor CD8 T cells were derived from $\beta_2M^{+/+}$ mice. It is unlikely that survival of memory CD8 T cells in $\beta_2M^{-/-}$ mice was due to the transferred CD8 T cells seeing MHC class I on each other because the transferred cells comprised only about 0.5 to 1% of the total splenocytes [$\sim 5 \times 10^5$ Thy1.1$^+$ CD8 T cells in (5 to 10) $\times 10^7$ $\beta_2M^{-/-}$ spleen cells]. Nevertheless, to test this possibility, we performed adoptive

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transfer experiments with MHC class I–deficient CD8 T cells obtained from chimeric mice ($\beta_2M^{-/-}$ bone marrow transferred to irradiated $\beta_2M^{+/+}$ mice) that had been immunized with LCMV. MHC class I–deficient memory CD8 T cells were able to persist in $\beta_2M^{-/-}$ mice (Fig. 1C). The $\beta_2M^{-/-}$ recipient mice were not irradiated before cell transfer because their "own" cells were transferred back into them. Thus, MHC class I– deficient memory CD8 T cells survive in $\beta_2M^{-/-}$ mice, and persistence of memory cells is seen after adoptive transfer into "full" (that is, nonirradiated) mice.

As a more stringent test for survival of memory T cells in the absence of MHC class I molecules, we next used $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice as recipients (*10*). LCMV-specific memory CD8 T cells (as well as total CD8 T cells) persisted indefinitely (>240 days) in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients. Similar to results seen with $\beta_2M^{-/-}$ mice (Fig. 1A), the initial expansion of LCMV-specific memory CD8 T cells was less in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice, but by day 20 there were similar numbers of memory CD8 T cells in both +/+ and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice. Figure 2B shows that LCMV-specific CD8 T cells can be identified by using MHC class I tetramers, and Fig. 2C documents proliferation of antigen-specific memory CD8 T cells and proliferation of total donor Thy1.1$^+$ CD8 T cells in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice. Taken together, these results show that even in mice completely lacking the classical MHC class I molecules (K^b and D^b) and β_2M, memory CD8 T cells can proliferate and survive for extended periods.

$D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice contain a few (~20 times lower than in +/+ mice) of their own CD8 T cells in the periphery (*11*), all of which are CD44^{hi} (*8*). We compared the turnover rate of these endogenous MHC class I–negative CD44^{hi} CD8 T cells with the cycling of the transferred memory CD8 T cells (Fig. 2C). The transferred memory CD8 T cells exhibited a pattern of cycling in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice [17 to 18% bromodeoxyuridine-positive (BrdU$^+$) cells] similar to that in +/+ mice (17 to 20% BrdU$^+$ cells), whereas the endogenous CD8 T cells of the −/− mice showed a distinctive pattern of hyperproliferation (61% BrdU$^+$ cells), with a large proportion of blasting cells (Fig. 2C) (*12*). These results show that proliferation of each of these populations is independently regulated, and that the cycling pattern of the "true" antigen-specific memory cells (generated in +/+ mice) remains the same in the presence or absence of MHC class I molecules.

A hallmark of memory T cells is their ability to exhibit rapid functional responses after reexposure to antigen (*2*, *4*). LCMV-specific memory CD8 T cells make interferon-γ (IFN-γ) within 4 hours after stimulation with the cognate peptide (*2*). In contrast, naïve CD8 T cells make IFN-γ after more than 24 hours, yet produce substantially lower amounts (*2*, *4*, *8*). We therefore examined whether memory CD8 T cells persisting in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice retain the ability to make rapid cytokine responses after reexposure to antigen and MHC class I, or revert to a naïve T cell phenotype. We found that this functional characteristic of memory CD8 T cells was retained indefinitely after transfer into $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice (Fig. 2D). The responsiveness of memory CD8 T cells transferred into −/− mice is identical to that of memory cells transferred into +/+ mice. Thus, after memory T cells are generated, they no longer need contact with MHC class I molecules to maintain their "response ready" mode. In addition, memory responses to multiple epitopes are maintained in the absence of MHC class I, and the epitope hierarchy is the same in +/+ and −/− mice (Fig. 2D).

Naïve CD8 and CD4 T cells require contact with MHC class I and class II molecules, respectively, for their maintenance in the periphery (*5*, *6*). Because our results (Figs. 1 and 2) showed that memory CD8 T cells can persist in MHC class I −/− mice, we directly compared MHC class I requirements of naïve and memory cells. Two series of experiments were done: one with monoclonal naïve and memory CD8 T

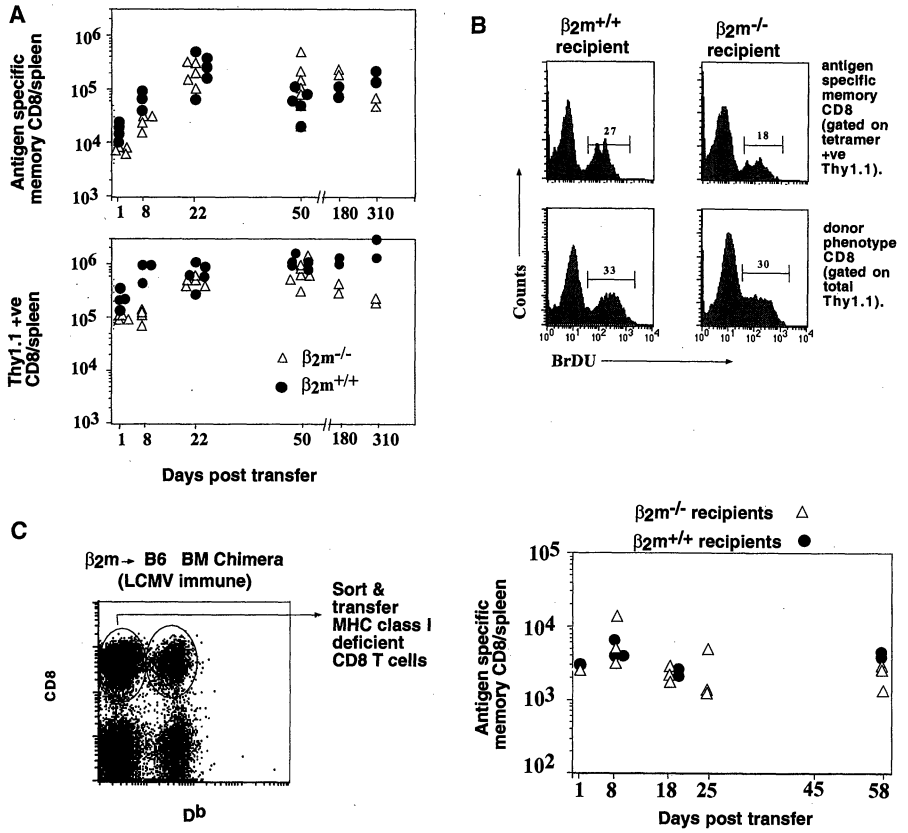


Fig. 1. Maintenance of memory CD8 T cells in MHC class I–deficient ($\beta_2M^{-/-}$) recipients. (**A**) FACS-purified Thy 1.1$^+$ CD8 T cells (1×10^6) containing 9×10^4 LCMV-specific memory CD8 cells were isolated from LCMV immune mice and transferred into irradiated [5.5 gray (Gy)] Thy1.2$^+$ $\beta_2M^{+/+}$ (●) and $\beta_2M^{-/-}$ (△) recipients (*21*). Data show the persistence of LCMV-specific memory CD8 (top) and total Thy1.1$^+$ CD8 (bottom) cells in both the recipients. (Top) Data represent total LCMV-specific memory CD8 T cells functionally responding to all of the five known epitopes by making IFN-γ (*2*). (**B**) Cycling of antigen-specific memory CD8 T cells in $\beta_2M^{-/-}$ and $\beta_2M^{+/+}$ mice as determined by BrdU incorporation. Seventy days after adoptive transfer of CD8 T cells, recipient mice were given BrdU in their drinking water for 1 week before analysis (*2*, *9*). BrdU staining is shown in MHC tetramers D^bNP396- and D^bG33-positive cells (top) and total Thy1.1$^+$ CD8 T cells (bottom). (**C**) Maintenance of MHC class I–deficient memory CD8 T cells in class I–deficient recipients. Mixed bone marrow chimeras were made by injecting 6×10^6 $\beta_2M^{-/-}$ and 2×10^6 $\beta_2M^{+/+}$ bone marrow cells into natural killer (NK) cell–depleted and lethally irradiated (8.5 Gy) C57BL/6 mice (*22*). After 1 month the chimeras were immunized with LCMV and rested for 6 months. The frequencies of LCMV-specific memory cells among MHC class I$^-$ and class I$^+$ CD8 cells in these immune chimeras were 1/15 and 1/14, respectively. FACS-sorted MHC class I–deficient CD8 T cells (5×10^5, left) (containing 3.4×10^4 LCMV-specific memory cells) were transferred into nonirradiated $\beta_2M^{-/-}$ and $\beta_2M^{+/+}$ recipients. $\beta_2M^{+/+}$ recipients were treated with antibody to NK1.1 to prevent NK-mediated rejection of class I–deficient donors (*22*). (Right) Persistence of class I–deficient memory CD8 T cells among recipients. CD8 T cells from recipient spleens were enriched by negative selection by using mouse CD8 subset column kit (R&D Systems, Minneapolis, Minnesota), and LCMV-specific memory CD8 frequency among enriched CD8 was determined by ELISPOT by stimulating with a mixture of five known LCMV CD8 epitope peptides (*2*).

cells expressing the T cell receptor (TCR) specific for LCMV peptide GP 33-41 (*13*), and the other with polyclonal CD44^{lo} (naïve) and CD44^{hi} (memory) CD8 T cells. Naïve transgenic CD8 T cells were unable to persist after transfer into $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice (Fig. 3A, bottom), and by day 40 there were 50- to 100-fold fewer cells in $-/-$ mice compared with $+/+$ mice. T cells expand after transfer into lymphopenic hosts (that is, irradiated, severe combined immunodeficient or RAG $-/-$ mice) (*5*, *14*). This "emptiness-induced" proliferation of naïve CD8 T cells was dependent on MHC class I molecules (Fig. 3A, top); although some proliferation was observed in $-/-$ mice at day 5 and 9, this was considerably less than that seen in $+/+$ mice and was not sustained. Our data also suggest that survival of naïve T cells is affected in the absence of MHC class I; a precipitous decline in cell numbers was observed between day 17 and 40. Maintenance is the sum of proliferation and survival, and taken together our results show that naïve CD8 T cells require interaction with MHC class I molecules not only for their proliferation after transfer into lymphopenic mice, but also for their long-term survival. In marked contrast to the behavior of naïve CD8 T cells, memory transgenic CD8 T cells persisted in $-/-$ mice, and at day 40 there was less than a twofold difference in the number of memory cells in $+/+$ versus $-/-$ mice (Fig. 3B). In addition, the proliferation of memory CD8 T cells after transfer into empty mice was only minimally affected, and by day 17 all of the memory cells had divided multiple times in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice (Fig. 3B). A similar pattern was seen with polyclonal CD44^{lo} (naïve) and CD44^{hi} (memory) CD8 T cells isolated from uninfected mice; proliferation of CD44^{lo} cells was compromised in $-/-$ mice, whereas proliferation of CD44^{hi} cells was independent of MHC class I molecules (Fig. 4). Memory cells divided at a faster rate than naïve cells even in $+/+$ mice.

In agreement with our findings, two recent studies with TCR transgenic CD8 T cells specific for the HY antigen showed that specific antigen is not necessary for memory maintenance. However, these studies reported that MHC class I molecules play a role in maintaining memory CD8 T cells (*6*). This conclusion differs from our findings and may reflect a unique characteristic of HY-specific T cells, differences in the experimental design, or both (*15*). It should be emphasized, however, that we have used the most extensive and stringent protocols for examining the requirement of MHC class I molecules in maintaining CD8 T cell memory. We have monitored the survival of several different populations of memory cells, including monoclonal and polyclonal antigen-specific CD8 T cells and total CD8 CD44^{hi} cells from both uninfected and immunized mice. Furthermore, in one series of experiments we used MHC class I–deficient memory CD8 T cells as donors. As recipients we used both irradiated and nonirradiated MHC class I–deficient mice. All of these experiments gave the same result, that is, that maintenance of CD8 T cell memory is independent of MHC class I molecules. This finding is in agreement with the observations of Swain *et al.* (*16*), who documented the persistence of memory CD4 T cells in MHC class II$^{-/-}$ mice. Thus, it appears that MHC independence is a property of both CD8 and CD4 memory.

The finding that memory CD8 T cells can maintain their functional characteristics and undergo proliferative renewal in the absence of MHC class I molecules does not necessarily

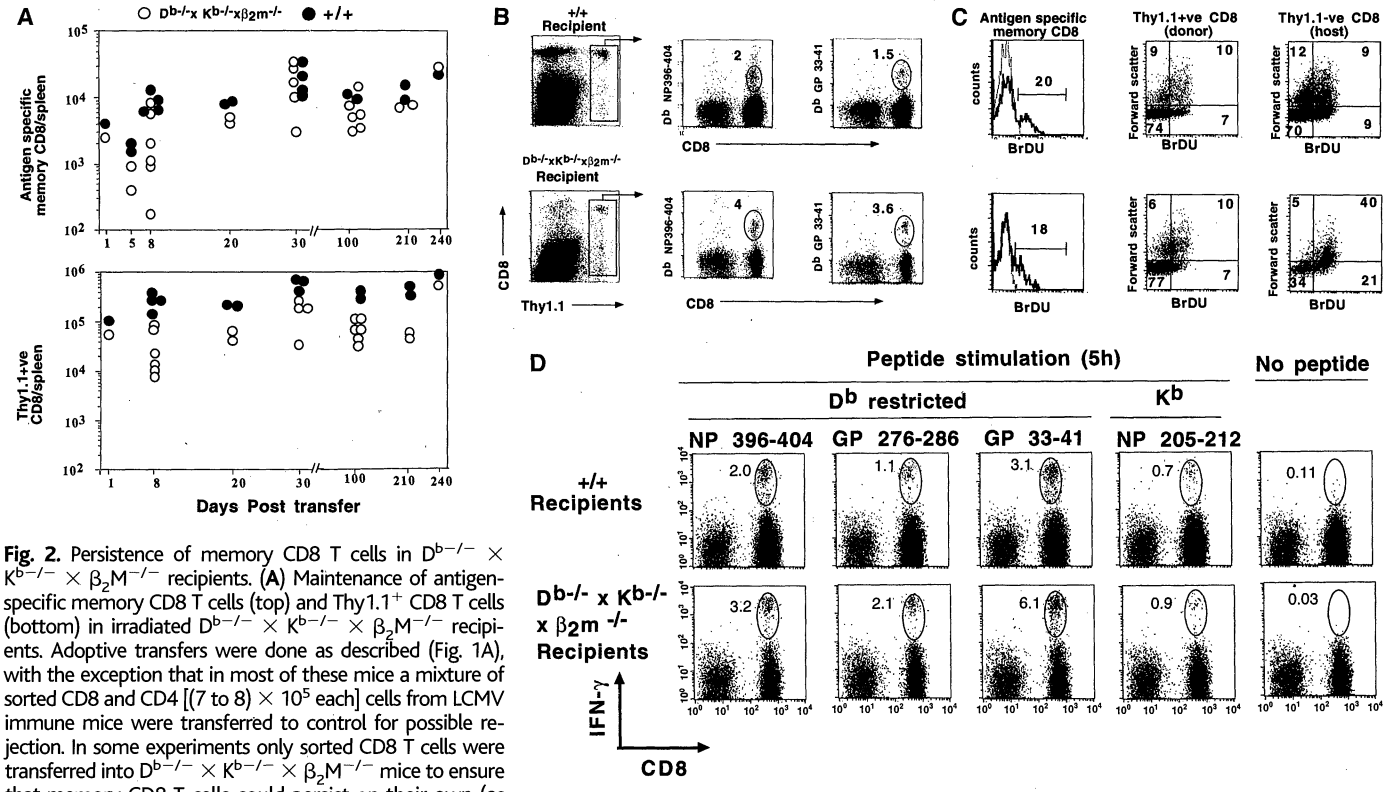


Fig. 2. Persistence of memory CD8 T cells in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients. (**A**) Maintenance of antigen-specific memory CD8 T cells (top) and Thy1.1$^+$ CD8 T cells (bottom) in irradiated $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients. Adoptive transfers were done as described (Fig. 1A), with the exception that in most of these mice a mixture of sorted CD8 and CD4 [(7 to 8) $\times 10^5$ each] cells from LCMV immune mice were transferred to control for possible rejection. In some experiments only sorted CD8 T cells were transferred into $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice to ensure that memory CD8 T cells could persist on their own (as seen in Fig. 1) after transfer into $\beta_2M^{-/-}$ mice. (**B**) Visualization of antigen-specific CD8 T cells in $+/+$ and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients at 100 days after transfer. The numbers indicate the percentage of Thy1.1$^+$CD8 that stain with the indicated MHC class I tetramer. (**C**) Cycling of antigen-specific (MHC tetramer DbGP33$^+$), Thy1.1$^+$, and endogenous CD8 cells in $+/+$ (top) and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ (bottom) recipients at 240 days after transfer. Three mice in each group were given BrdU-containing water for 1 week before analysis, and their spleens were pooled and analyzed. Dotted line represents staining of the same cells with isotype control for anti-BrdU. (**D**) Rapid functional response to different epitopes by memory CD8 T cells obtained from $+/+$ and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients at 100 days after transfer. Spleen cells from $+/+$ and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients were stimulated with different peptides as described (*21*). Data represent events gated on Thy1.1$^+$ cells. Numbers indicate the percentage of Thy1.1$^+$ CD8 that score positive for intracellular IFN-γ.

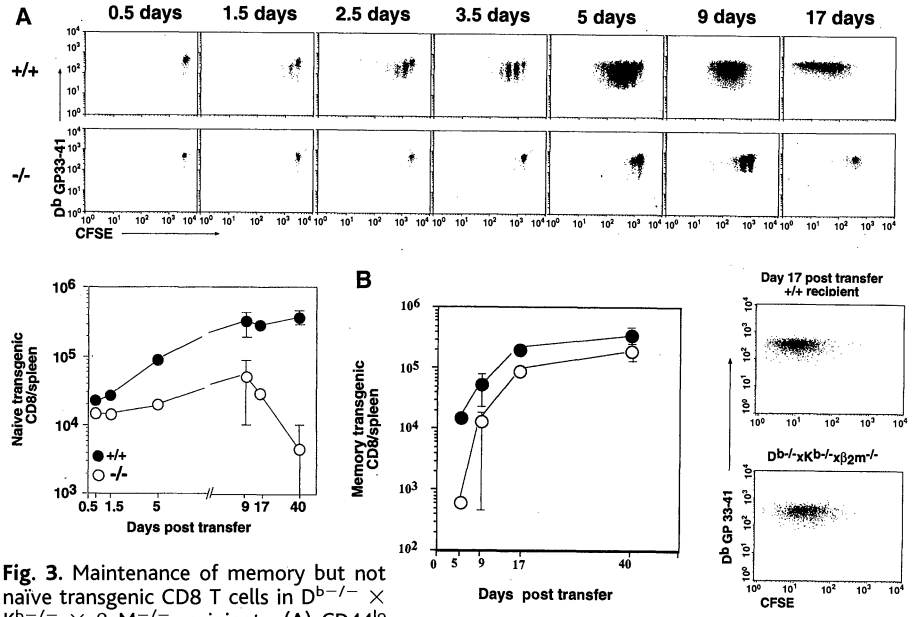
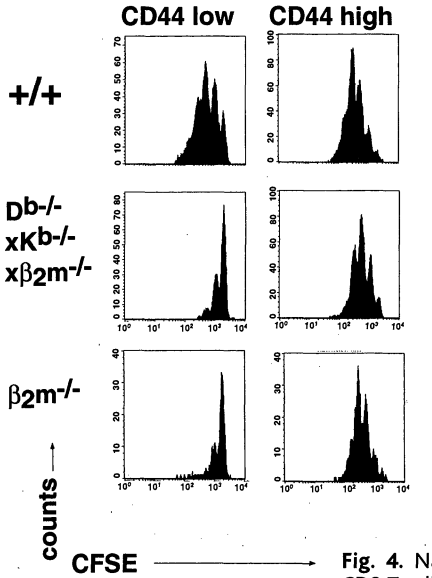


Fig. 3. Maintenance of memory but not naïve transgenic CD8 T cells in $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients. (**A**) $CD44^{lo}$ CD8 T cells from naïve P-14 transgenic mice expressing the TCR specific to LCMV peptide GP 33-41 were sorted, labeled with fluorescent dye CFSE (*23*), and transferred into irradiated recipients. The transferred population contained >95% transgenic cells as assessed by MHC tetramer D^bGP33 staining, anti-$V_\alpha2$ and anti-$V_\beta8.1$ staining, or both. (Bottom) The total number of MHC tetramer D^bGP33^+ CD8 T cells (average of two to four mice at each time point) and (top) the number of cell divisions as seen by fluorescent dye dilution in +/+ and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ (−/−) recipients at various time points after transfer. (**B**) Memory P-14 transgenic CD8 T cells were made as follows: Fifty thousand CD8 T cells from naïve P-14 transgenic mice were transferred intravenously into C57BL/6 mice. After 2 days mice were immunized with 2×10^5 PFU of LCMV and rested for more than 6 months. Forty percent of $CD44^{hi}$ CD8 cells from these immune mice were positive for MHC tetramer D^b GP 33-41, $V_\alpha2$, and $V_\beta8.1$. These cells also exhibit other memory cell characteristics described in (*21*). CD8 $CD44^{hi}$ cells from these immune transgenic chimeras at 6 months after transfer were sorted and transferred into +/+ and $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ recipients as described above. Data represent the total number of tetramer-positive memory CD8 T cells at various days after transfer (left) (average of two to four mice) and the number of divisions among tetramer-positive cells on day 17 after transfer (right).



CD44 low

Recipient mice	% cells at each division number					
	0	1	2	3	4	5
++	10	24	34	21	8	2
$D^{b-/-}K^{b-/-}\beta_2m^{-/-}$	56	35	8	0.7	0	0
$\beta_2m^{-/-}$	54	34	6	2	1	0.4

CD44 high

Recipient mice	% cells at each division number					
	0	1	2	3	4	5
++	2	10	31	39	14	2
$D^{b-/-}K^{b-/-}\beta_2m^{-/-}$	6	22	44	26	3	0.4
$\beta_2m^{-/-}$	2	10	33	41	11	0.8

Fig. 4. Naïve ($CD44^{lo}$) and memory ($CD44^{hi}$) polyclonal CD8 T cells from uninfected mice behave similarly to naïve and memory CD8 T cells of known specificity. $CD44^{lo}$ and $CD44^{hi}$ CD8 T cells from $Thy1.1^+$ donors were sorted, labeled with CFSE, and transferred into irradiated +/+, $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$, and $\beta_2M^{-/-}$ recipients. The CFSE fluorescence levels among $Thy1.1^+$ CD8 T cells are shown on day 5 after transfer. The percentage of cells at each division number is shown in the table.

rule out a role for the TCR in cell survival and proliferation. It is possible that intrinsic constitutive signals from the TCR may be necessary for the maintenance of T cells (*17*). Recent studies with the cre-lox system have shown that the B cell receptor (immunoglobulin) is required for survival of B cells (*18*). Similar studies with T cells will be necessary to directly assess the contribution of the TCR.

Why do naïve CD8 T cells differ from memory CD8 T cells in terms of their requirements for MHC class I molecules? It is conceivable that the topology of the signaling machinery (for example, TCR, CD8 coreceptor, adhesion molecules, and kinases) is sufficiently different between naïve and memory cells such that memory cells can still receive the necessary signals in the absence of contact with MHC, whereas naïve cells require MHC interaction for their maintenance. Recent studies have shown changes in the organization of molecules at the cell surface, as well as changes in the chromatin organization and gene expression pattern, of memory compared with naïve cells (*19*). A combination of these changes may be required to generate a memory cell that is independent of MHC class I.

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10. $D^{b-/-}$ and $K^{b-/-}$ mice [S. Pascolo *et al.*, *J. Exp. Med.* **185**, 2043 (1997)] were back-crossed to each other and then to $\beta_2M^{-/-}$ mice to obtain $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice. All of the mice were on a C57BL/6 background.
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12. This hyperproliferation of endogenous CD8 T cells of $D^{b-/-} \times K^{b-/-} \times \beta_2M^{-/-}$ mice is unlikely to be due to stimulation with MHC class I present on the transferred CD8 T cells because even in $-/-$ mice that have not received any cell transfer, the endogenous CD8 T cells exhibit a similar pattern of hyperproliferation (*8*).
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15. Although Tanchot *et al.* (*6*) reached a different conclusion, there are many similarities between their study and ours. In agreement with us, Tanchot *et al.* (*6*) found that naïve CD8 T cells were more dependent on MHC class I molecules than memory T cells. For example, they showed that HY-specific memory CD8 T cells (which recognize HY peptide presented by D^b) were able to expand in $D^{b-/-}$ mice, whereas naïve CD8 T cells disappeared. Similar to our finding, they also reported proliferation of HY-specific memory CD8 T cells in MHC class $I^{-/-}$ ($D^{b-/-} \times \beta_2M^{-/-}$) mice. However, in contrast to our results, they found that despite this proliferation, HY-specific memory CD8 T cells declined (>20-fold drop in 2 weeks) in MHC class $I^{-/-}$ mice. A potential complication of the Tanchot *et al.* (*6*) study was that they transferred HY-specific memory CD8 T cells from B6 mice into $D^{b-/-} \times \beta_2M^{-/-}$ mice that were on a 129 × B6 background. It is well established that mice with 129 background can pose problems in adoptive transfer experiments and in fact when we used the same mice that Rocha used in her study neither CD8 nor CD4 T cells from B6 mice survived after adoptive transfer (*20*). However, new preliminary experiments with MHC class $I^{-/-}$ mice backcrossed to B6 show the same results with HY transgenic memory T cells (B. Rocha, personal communication). Thus, it is possible that differences between our findings may reflect a unique property of the HY-specific transgenic CD8 T cells. Consistent with this is the observation that unlike most naïve CD8 T cells (either transgenic or polyclonal), naïve HY transgenic T cells do not divide even in +/+ lymphopenic mice. The study by Markiewicz *et al.* (*6*) is difficult to compare with ours because they examined the survival of in vitro–activated HY-specific effector and naïve CD8 T cells after transfer into $TAP^{-/-}$ mice. They found that the activated cell population was more dependent on MHC class I for their survival than naïve CD8 T cells. Thus, it is conceivable that survival of CD8 effectors (which are highly susceptible to apoptosis), and not memory CD8 T cells, was being analyzed in their study.
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21. Thy1.1$^+$ C57BL/6 mice were immunized with 2×10^5 PFU of LCMV Armstrong and rested for 3 to 8 months. Spleen cells from groups of these mice were pooled, stained for surface CD8, sorted in a FACSVantage (Becton Dickinson), and transferred intravenously into irradiated (550R) Thy1.2 recipients. Before transfer, LCMV-specific memory CD8 T cell numbers were determined by staining with MHC class I tetramers and by single-cell assays (ELISPOT and intracellular stain) measuring IFN-γ production (*2*). After adoptive transfer, the donor CD8 T cells were identified by the Thy1.1 marker, and LCMV-specific memory cells were quantitated with MHC class I tetramers and by cytokine assays (*2*). Memory cells transferred into MHC class I–deficient mice were analyzed for cytokine production by adding spleen cells from uninfected +/+ mice (1:1 ratio) to present the peptide. LCMV-specific memory CD8 T cells were distinguished from effector cells on the basis of minimal to no ex vivo cytolytic activity (>20-fold lower than effector cells on a per cell basis), low levels of CD69, low IL-2Rα (CD25), and low transferrin receptor (CD71) expression. They also differed from naïve cells on the basis of high expression of cell surface markers CD44 and Ly6C and their ability to induce rapid cytokine responses.
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Class II–Independent Generation of CD4 Memory T Cells from Effectors

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The factors required for the generation of memory CD4 T cells remain unclear, and whether there is a continuing requirement for antigen stimulation is critical to design of vaccine strategies. CD4 effectors generated in vitro from naïve CD4 T cells of mice efficiently gave rise to small resting memory cells after transfer to class II–deficient hosts, indicating no requirement for further antigen or class II recognition.

Signals through the T cell receptor (TCR), provided by high doses of peptide antigen bound to class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs), are critical for the activation of naïve CD4 T cells and for their transition to effector and memory cells (*1*). However, transfer of effectors, generated in vitro from naïve CD4 T cells, to adoptive hosts results in the development of long-term CD4 memory even though no further antigen is introduced (*2*), contradicting some studies stressing the importance of antigen persistence (*3*), but agreeing with others arguing against such a role (*4*). Different strengths of TCR interaction with peptide-MHC are needed for T cell response at different stages of T cell differentiation and can induce different outcomes. In the thymus, low-avidity TCR interaction with self peptides bound to self MHC induces positive selection (*5*). Naïve CD8 T cells in class I–deficient hosts (*6*) and naïve CD4 T cells in class II–deficient mice (*7, 8*) have shortened life-spans, suggesting that interactions between TCR and MHC prolong naïve T cell life-span. Naïve CD4 T cells require high avidity/density TCR interactions for induction of cytokine synthesis, whereas effector and memory cells respond efficiently at lower avidity/density (*9*). Thus, effector and memory cells might be expected to overcome this MHC dependence. However, in two recent studies, activated CD8 T cells did not generate long-term memory after transfer to class I–deficient hosts (*10*). Using a model where effectors are generated in vitro and then transferred to adoptive hosts, which do or do not express class II, we found that neither antigen recognition nor interaction with class II is necessary for the generation of memory or for its persistence.

T helper cell 1 (T_H1) or T_H2 cytokine-polarized effectors were generated in vitro from naïve CD4 T cells of AND TCR transgenic (Ig) mice (*11*) by stimulation with PCCF (a fragment of pigeon cytochrome) and mitomycin-treated I-E^k transfected fibroblast, DCEK-ICAM, or T cell–depleted APC from B10.BR mice (*2, 9*). The added APCs are no longer present in the cultures after 24 to 48 hours (*12*). In aged AND mice, Tg^+ memory cells do not develop, suggesting a lack of environmental antigens capable of stimulating cross-reacting responses (*13*). The effectors generated are >99% $CD4^+$, Tg^+ cells and contain no detectable APCs or APCs capable of mediating their restimulation (*14*).

To evaluate the possibility that endogenous TCR chains could contribute to memory generation or persistence, we crossed the AND mice to RAG-2–deficient mice [RAG knockout (KO)] (*15*). Effector cells were generated from naïve CD4 T cells, and aliquots were transferred to T cell–deficient hosts (*16*) created by adult thymectomy, lethal irradiation, and bone marrow reconstitution (ATXBM) (*17*). Both donor and recipient were on a B6 background (*17*), making allogeneic reactions unlikely. Because the hosts are devoid of T cells, there is ample opportunity for transferred cells to receive other, potentially important, non–TCR mediated signals. Equivalent numbers of Tg^+ CD4 T cells were seen in hosts 3, 8, and 13 weeks after transfer. The recovered cells were small, $CD44^{hi}$ with a memory phenotype (*17*). Equal numbers of Tg^+ recovered memory cells were restimulated ex vivo, and cytokine titers in the supernatants were determined (*18*). The cytokine pro-

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