transcription (*1*, *23*). Although it has not yet been shown that phosphorylation of these transcription factors is responsible for elicitor-induced transcription of PR genes, the elicitor-induced relocation of ERM kinase into the nucleus might link cytosolic signal transduction to nuclear activation of plant defense genes.

MAP kinases were first found in yeast and animals, where they participate in signaling cascades linking plasma membrane receptors that perceive extracellular signals to a variety of cellular response mechanisms (*24*, *25*). The MAP kinases known in plants are activated by environmental stresses and plant hormones (*26*, *27*). Our results demonstrate posttranslational and transcriptional activation of a plant MAP kinase within a signal transduction pathway that mediates the response to a pathogen. Activation of ERM kinase follows input from receptor-regulated ion channels of the plasma membrane and precèdes or parallels the formation of O_2^- radicals, which in turn activate defense genes and phytoalexin synthesis (*17*).

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Differential Requirements for Survival and Proliferation of CD8 Naïve or Memory T Cells

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The requisite molecular interactions for CD8 T cell memory were determined by comparison of monoclonal naïve and memory $CD8^+$ T cells bearing the T cell receptor (TCR) for the HY antigen. Naïve T cells required only the right major histocompatibility complex (MHC) class I–restricting molecule to survive; to expand, they also needed antigen. In contrast, for survival, memory cells did not require the restricting MHC allele, but needed only a nonspecific class I; for expansion the correct class I, but not antigen, was required. Thus, maintenance of CD8 T cell memory still required TCR–MHC class I interactions, but memory T cells may have a lower functional activation threshold that facilitates secondary responses.

The molecular basis of T cell memory remains elusive (*1*, *2*). It is not known if memory responses depend exclusively on an increased frequency of antigen-specific T cells (*3*) or if "memory T cells" with novel biological capacities are generated (*4*). Memory responses have been reported to depend on continuous antigenic stimulation (*5*), but others have observed the persistence of increased frequencies of antigen-specific $CD8^+$ T cells in the apparent absence of antigen (*6*–*8*).

We have investigated the conditions necessary in vivo for the survival and expansion of naïve and memory antigen-specific $CD8^+$ T cells. Because of the degeneracy and redundancy of T cell receptor (TCR) usage in most immune responses, individual clones of antigen-specific T cells "in vivo" cannot be easily examined. T cells may also coexpress different TCRs, and their behavior may be conditioned by nonspecific antigen effects (*1*, *2*). Thus, to characterize the functional properties and the requirements for persistence of memory T cells, we used monoclonal T cell populations.

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Transgenic (Tg) mice bearing a Tg αβ TCR specific for the HY male antigen restricted to major histocompatibility complex (MHC) class I $H\text{-}2D^b$ and deficient in the recombinase gene *RAG2* ($TgRAG2^-$) (*9*) were used to obtain monoclonal populations of $CD8^+$ T cells. In female $TgRAG2^-$ mice, all T cells positively selected in the thymus are $CD8^+$ Tg $TCR\alpha\beta^+$ (Fig. 1A). These cells represent a pure population of naïve T cells, because cross-reactivity with environmental antigens cannot be detected: All these cells are $CD44^-$ and do not divide (*10*, *11*). Studying these cells ex vivo, we could not detect lymphokine mRNAs, but these could be induced after in vitro stimulation with monoclonal antibodies (mAbs) to CD3 (anti-CD3). Virgin T cells constitutively expressed little perforin and FasL mRNAs, which were up-regulated after anti-CD3 stimulation (Fig. 1B) (*12*).

To study the TCR interactions required for the survival or division of naïve CD8 T cells, we compared their fate after transfer into irradiated hosts (*13*) that differed in MHC class I and HY antigen expression. These hosts were C57BL/6 CD8-deficient (*14*) male ($HY^+H\text{-}2^{b+}$) and female ($HY^-H\text{-}2^{b+}$) mice; female $H\text{-}2D^b$-deficient mice (*15*) that lack the MHC class I restriction element of this Tg TCR (*16*) but express other MHC class I molecules including $H\text{-}2K^b$ ($HY^-H\text{-}2D^{b-}$ class I^+); and female class I^- mice defi-

cient in both H-2D^b and β_2-microglobulin (HY$^-$D$^b-\beta_2$M$^-$). These mice were used rather than β_2M-deficient mice, because the latter mice express H-2D^b (*17*)—enough to induce deletion of male-specific Tg T cells in male mice (*18*). To correlate cell survival with interactions with MHC class I–restricting element, we used female mice expressing H-2D^b but lacking H-2K^b (HY$^-$D^{b+}K^{b-}). These host mice were irradiated (*13*) and injected 2 days later with 10^6 naïve cells. Recovery of Tg cells was evaluated at days 1, 2, 7, and 13 after transfer (*19*). We studied Tg T cell division 1 week after transfer by monitoring bromodeoxyuridine (BrdU) incorporation.

One day after cell transfer, the fraction of donor cells homing to the pool of lymphoid organs studied was the same in all groups of host mice (about half of the donor cell population) (*20*). Naïve T cells could survive in a resting state in female CD8-deficient mice (Fig. 1, C and D); they did not incorporate BrdU (*21*), and the number recovered was constant from day 1 up to 2 weeks after injection. Expansion of naïve cells required stimulation with male antigen (*22*) because they divided only after transfer into male CD8-deficient hosts. Survival of naïve T cells required the right MHC-restricting element. In mice lacking H-2D^b or expressing no class I (H-2D$^{b-}\beta_2$M$^-$), naïve cells did not survive, but decayed to an average of 3% of the injected cohort at 1 week, 1% at 13 days (Fig. 1D and Table 1), and were undetectable at 2 weeks. This decay correlated with the absence of interactions with the MHC restriction element, because naïve H-2D^b–restricted Tg cells persisted after transfer into H-2K^b–deficient mice expressing H-2D^b (below). Thus, as described during thymus positive selection, a minimal state of cell activation may allow survival in the absence of cell division (*23*).

We next studied the TCR interactions required to maintain CD8$^+$ T cell memory. To obtain memory cells, we stimulated female naïve Tg T cells with relatively low doses of male antigen in vivo in male→female bone marrow (BM) chimeras (*24*). We produced male→female B mice by injecting a mixture of 90% female and 10% male BM cells from CD3ε-deficient mice into RAG2-deficient female mice (*25*). The hosts did not have endogenous T cells, and 10% of BM-derived cells were of male origin (Fig. 2A, left).

Naïve T cells transferred into these chimeras expanded. In chimeras injected with 0.5×10^6 naïve T cells, 15×10^6 to 20×10^6 T cells could be recovered 1 month later and for at least 7 months. These cells were able to eliminate the antigen in vivo, as shown by the disappearance of male BM-derived cells (Fig. 2A, middle). These antigen-experienced Tg T cells proliferated in response to the male antigen "in vitro" (8) and maintained the capacity to mediate effector functions in vivo, because they eliminated male BM-derived cells when transferred into new set of male→female BM chimeras (Fig. 2A, right). As described in other systems (*6*, *7*), memory cells in these chimeras appeared to survive in the

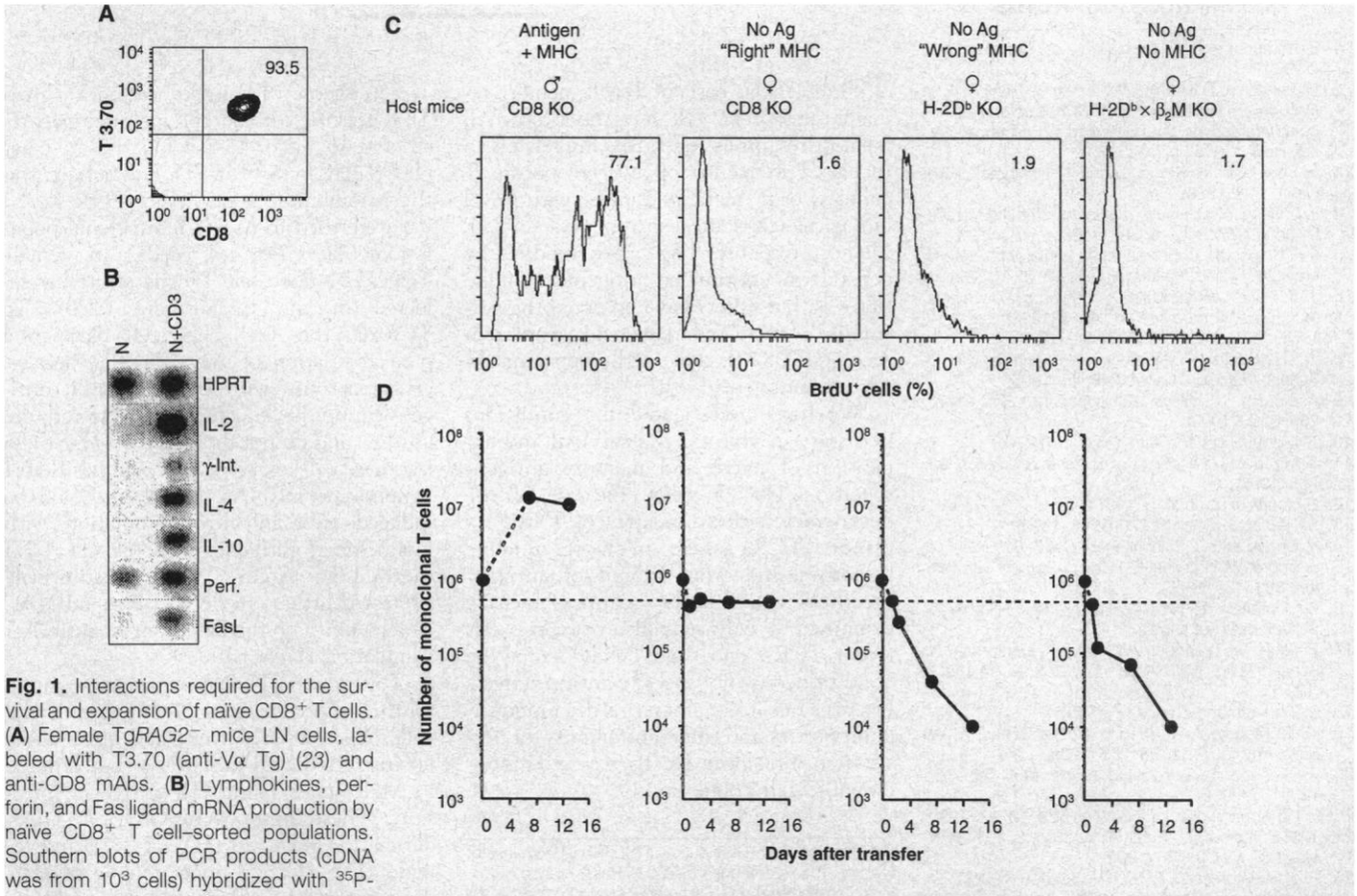

Fig. 1. Interactions required for the survival and expansion of naïve CD8$^+$ T cells. (**A**) Female Tg*RAG2*$^-$ mice LN cells, labeled with T3.70 (anti-Vα Tg) (*23*) and anti-CD8 mAbs. (**B**) Lymphokines, perforin, and Fas ligand mRNA production by naïve CD8$^+$ T cell–sorted populations. Southern blots of PCR products (cDNA was from 10^3 cells) hybridized with ^{35}P-labeled–specific probes are shown. Negative samples were also tested with 10 times the concentration of cDNA, and 35 cycles of amplification. This also failed to reveal mRNA synthesis. (**C** and **D**) Irradiated (600 R) host mice were injected with 10^6 naïve Tg cells. (C) Histograms showing BrdU incorporation of T3.70$^+$ CD8$^+$ T cell–sorted populations, recovered at day 7 after transfer from different host mice injected for 3 days with BrdU. Background BrdU labeling (in mice not injected with BrdU) averaged 2%. (D) Absolute numbers of Tg cells recovered at days 1, 2, 7, and 13. The horizontal dashed line shows the number of cells that home to the lymphoid organs studied 1 day after cell transfer.

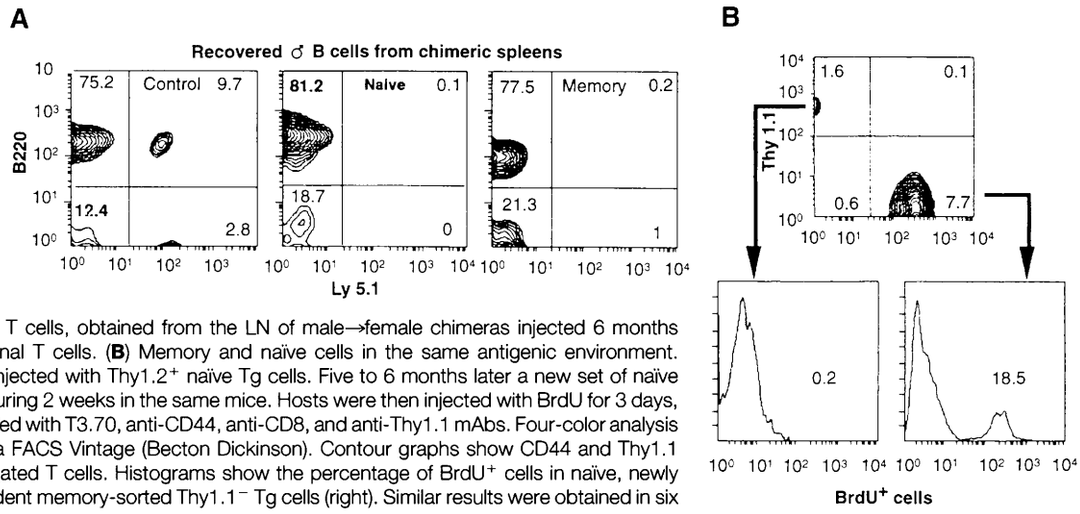

Fig. 2. Tg cells eliminated male antigen-expressing cells in vivo. (**A**) The spleen of male→female chimeras, labeled with anti-B220 and anti-Ly5.1 mAbs. The fraction of $B220^+Ly5.1^+$ indicates the percentage of male B cells present in host mice. Labeling in the absence of T cells (left), 1 month after injection of 5×10^5 LN naïve Tg T cells (middle), and 5×10^5 memory Tg T cells, obtained from the LN of male→female chimeras injected 6 months previously with naïve monoclonal T cells. (**B**) Memory and naïve cells in the same antigenic environment. Male→female chimeras were injected with $Thy1.2^+$ naïve Tg cells. Five to 6 months later a new set of naïve $Thy1.1^+$ Tg cells was parked during 2 weeks in the same mice. Hosts were then injected with BrdU for 3 days, and cell suspensions were labeled with T3.70, anti-CD44, anti-CD8, and anti-Thy1.1 mAbs. Four-color analysis and cell sorting were done in a FACS Vintage (Becton Dickinson). Contour graphs show CD44 and Thy1.1 expression of $CD8^+$ $T3.70^+$–gated T cells. Histograms show the percentage of $BrdU^+$ cells in naïve, newly injected $Thy1.1^+$ (left), and resident memory-sorted $Thy1.1^-$ Tg cells (right). Similar results were obtained in six mice.

absence of antigen. Because no male cells were detected in the spleen (Fig. 2A), lymph nodes (LNs), or BM (8), when a new set of naïve cells were parked for 2 weeks in these mice, the cells retained the $CD44^-$ naïve phenotype and did not divide (Fig. 2B). Six months after the apparent elimination of antigen, resident memory cells were still cycling (26) because 20 to 30% incorporated BrdU after a 3-day discontinuous pulse (Fig. 2B) (19). Therefore, naïve and antigen-experienced cells behaved differently in the same host antigenic environment, suggesting that they may differ in their requirements for survival and stimulation. As evaluated by CD44, CD25, and CD69 expression (Fig. 3A), antigen-experienced Tg cells have the memory T cell phenotype (26). They constitutively expressed mRNA encoding interleukin-2 (IL-2) and interferon-γ (IFN-γ), FasL, and perforin, the latter two to a larger extent than naïve cells. This pattern of mRNA expression was stable and identical to that detected in $CD44^+$ non-Tg $CD8^+$ T cells (Fig. 3B).

To identify the TCR interactions required for the survival or division of memory CD8 T cells, we injected purified (>97%) memory populations [depleted of B cells and other class II–positive antigen-presenting cells (APCs)] into irradiated hosts (13) that differed in MHC class I or antigen presentation (16). Transgenic T cell recovery was determined at days 1, 2, 7, and 11 after transfer, whereas T cell division was evaluated by BrdU incorporation

Table 1. Recovery of Tg cells, 1 week after transfer into various irradiated hosts, that differed in MHC class I or antigen presentation. Mice were injected with 1×10^6 Tg cells. Results represent the absolute number of Tg cells ($\times 10^{-5}$) recovered in host mice and are the mean of two experiments (male mice) or the mean ± SE of three to five independent experiments in other mice (two mice per experiment). Mice injected with naïve and memory cells were studied simultaneously. KO, knockout.

♂ CD8 KO	♀ CD8 KO	♀ H-2D^b KO	♀ H-2D^b × β_2M KO
		Naïve	
134.5	4 ± 0.6	0.3 ± 0.1	0.7 ± 0.3
		Memory	
166.1	18.1 ± 2.3	6.6 ± 1.5	1.0 ± 0.3

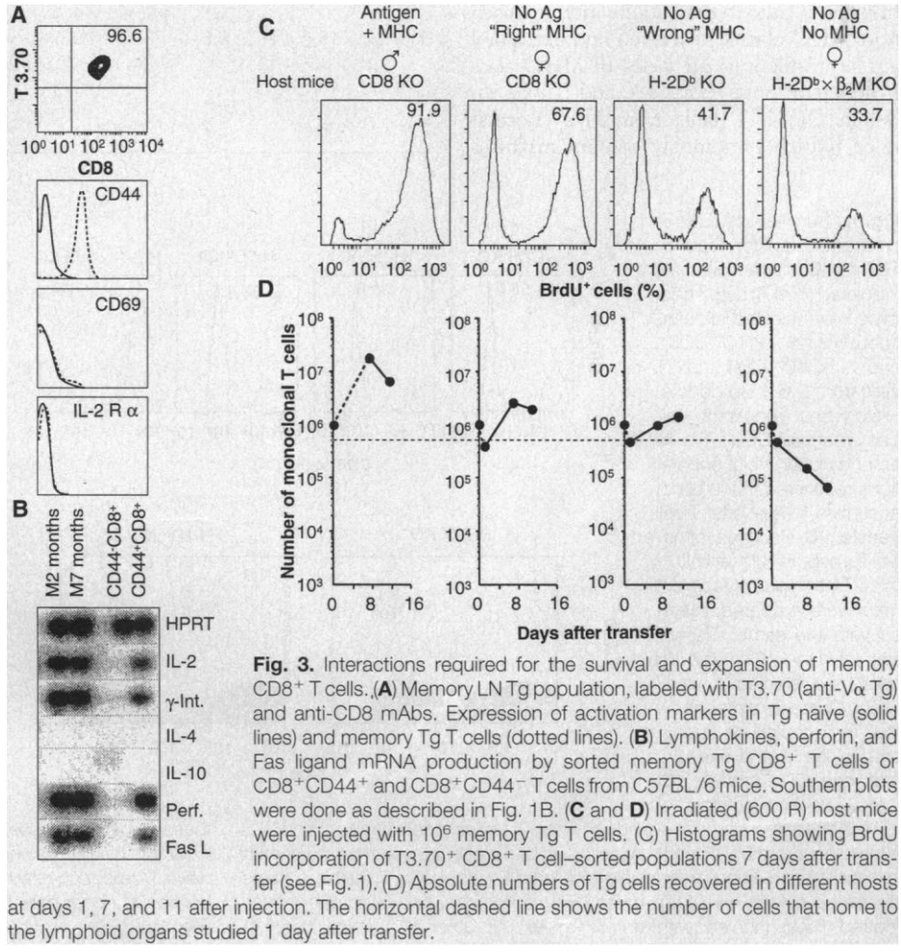

Fig. 3. Interactions required for the survival and expansion of memory $CD8^+$ T cells. (**A**) Memory LN Tg population, labeled with T3.70 (anti-Vα Tg) and anti-CD8 mAbs. Expression of activation markers in Tg naïve (solid lines) and memory Tg T cells (dotted lines). (**B**) Lymphokines, perforin, and Fas ligand mRNA production by sorted memory Tg $CD8^+$ T cells or $CD8^+CD44^+$ and $CD8^+CD44^-$ T cells from C57BL/6 mice. Southern blots were done as described in Fig. 1B. (**C** and **D**) Irradiated (600 R) host mice were injected with 10^6 memory Tg T cells. (C) Histograms showing BrdU incorporation of $T3.70^+$ $CD8^+$ T cell–sorted populations 7 days after transfer (see Fig. 1). (D) Absolute numbers of Tg cells recovered in different hosts at days 1, 7, and 11 after injection. The horizontal dashed line shows the number of cells that home to the lymphoid organs studied 1 day after transfer.

at day 7 (Fig. 3, C and D, and Table 1).

At 24 hours after transfer, homing of memory Tg cells was the same in all groups of host mice and similar to that of naïve Tg cells (Fig. 1). When memory Tg cells were stimulated after transfer into male $CD8^-$ mice, their rate of division (>90% $BrdU^+$ cells) was higher than that of naïve cells. In contrast to naïve cells, memory cells transferred into female $CD8^-$ hosts also divided extensively (70% $BrdU^+$) (*27*) and survived and divided in mice lacking the H-$2D^b$–restricting element (42% $BrdU^+$). In mice lacking class I (H2-$D^{b-}\beta_2M^-$ mice), about 30% of memory cells still incorporated BrdU, indicating a response to autocrine or environmental growth factors, even in the absence of T cell stimulation (*26*). However, this response was not sufficient to maintain memory T cells, which disappeared progressively (Fig. 3, C and D, and Table 1). Two weeks after T cell transfer, donor cells in class I–deficient (H-$2D^{b-}\beta_2M^-$) host mice were barely detectable.

The disappearance of Tg T cells after transfer into MHC-deficient irradiated hosts was not due to natural killer cell activity or T cell–mediated graft rejection by the irradiated hosts (Fig. 4). $CD4^+$ cells from H-2^b mice (that could interact with host MHC class II) survived and expanded after transfer into all types of MHC class I–deficient hosts (Fig. 4A and Table 2). When $CD8^+$ T cells from H-2^b normal mice (which presumably contain mixtures of H-$2K^b$– and H-$2D^b$–restricted naïve and memory cells) were transferred, they survived and expanded in both H-$2D^{b-}$ and H-$2K^{b-}$ hosts and decayed only after transfer into class I^- ($D^{b-}\beta_2M^-$) hosts (Fig. 4A and Table 2) (*28*). These experiments demonstrate that the survival of transferred $CD8^+$ T cells correlated with their requirement for TCR–MHC class I interactions, because if cell decay were due to cell rejection, all donor cells (that express both H-$2D^b$ and H-$2K^b$) should be recognized and eliminated in all types of MHC-deficient host mice. These results also suggested that normal $CD8^+$ T cells, like Tg lymphocytes, also require TCR–MHC class I interactions to survive in the periphery.

Table 2. Recovery of B6 Ly5.1 T cells, 1 week after transfer into various irradiated hosts. Host mice were injected simultaneously with 1×10^6 $CD8^+$ and 2×10^6 $CD4^+$ LN T cells from $Ly5.1^+$ B6 donors. Results represent the absolute numbers ($\times 10^{-5}$) of $CD8^+$ and $CD4^+$ donor T cells recovered in the different hosts in a single experiment. Similar results were obtained in three other experiments (*37*).

♀ CD8 KO	♀ H-$2K^b$ KO	♀ H-$2D^b$ KO	♀ H-$2D^b \times \beta_2M$ KO
		$CD4^+$	
9	15.5	8.5	28.6
		$CD8^+$	
7.1	6.6	5.7	1.5

Because in the above experiments all transferred B6 populations may have undergone expansion whereas naïve cells remained resting, the results could be biased if host mice would preferentially eliminate resting donor cells. To exclude this possibility, we compared the survival of naïve Tg T cells after transfer into female D^{b-} and K^{b-} mice, in which naïve cells do not divide (Fig. 4, B and C). K^{b-} hosts were also selected according to the levels of D^b expression (*29*). In K^{b-} hosts expressing normal amounts of D^b, the recovery of naïve donor cells was the same as in class I–bearing recipients. In K^{b-} mice with a reduced expression of D^b, cell recovery was lower, but still higher than that observed in D^{b-} mice (Fig. 4C). Therefore, the frequency of surviving naïve T cells recovered in the female recipient mice was related to the level of class I MHC expression, as described during thymus positive selection (*30*).

The disappearance of memory $CD8^+$ cells in a class I^- environment disagrees with previous findings describing the survival of memory T cells in β_2M^- hosts (*6*). These differences may be due to the expression of MHC class I in β_2M^- mice (*17*), which interferes with T cell repertoire selection (*18*), or to the high number of T cells injected, or to both factors: in these instances it is likely that donor cells expressing class I interact among themselves in the host environment.

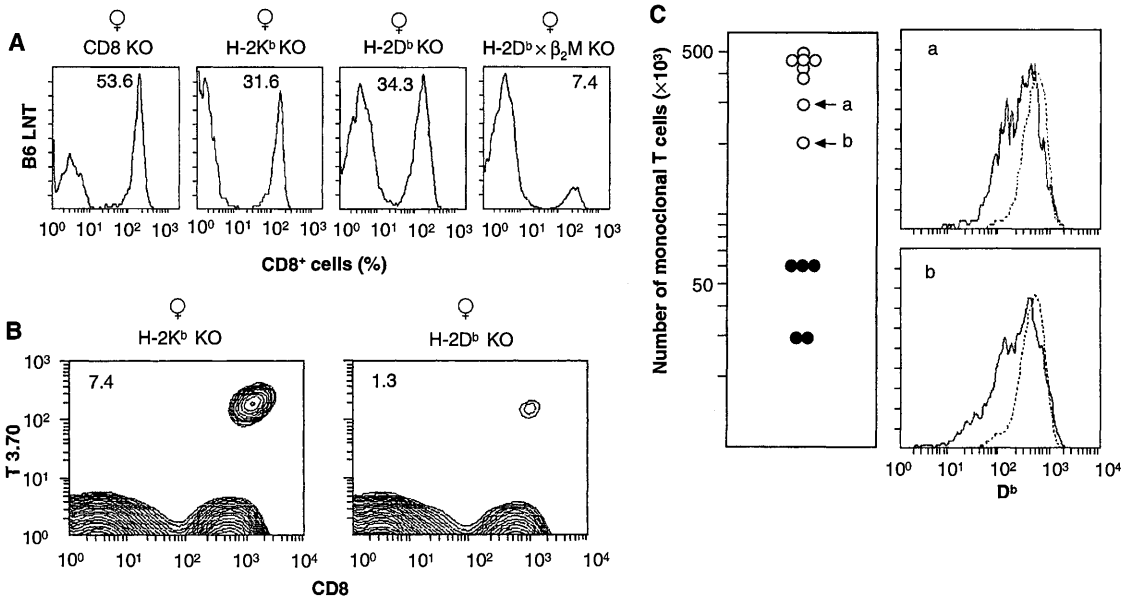

Fig. 4. Survival of $CD8^+$ T cells after transfer requires TCR-MHC interactions. (**A**) Irradiated (600 R) Ly5.2 host mice were injected simultaneously with 1×10^6 $CD8^+$ and 2×10^6 $CD4^+$ LN T cells from Ly5.1 B6 donors. Histograms represent the percentage of $CD8^+$ T cells among donor T cell populations recovered in the LN of host mice 1 week after T cell transfer. (**B**) Recovery of naïve Tg cells in K^{b-} and D^{b-} mice. Both types of recipient mice were irradiated, injected with the same suspension of 1×10^6 naïve Tg cells, and studied 1 week later. Contour plots show the Tg populations recovered in the LNs of one K^{b-} and one D^{b-} mouse. (**C**) (Left) Absolute number of naïve Tg cells recovered in individual mice. K^{b-} mice (open symbols) and D^{b-} mice (closed symbols). In K^{b-} mice (a and b), Tg cell recovery was lower than in class I^+ mice. These mice down-regulated D^b, as shown in the histograms displaying spleen cells from these mice (solid lines) and a normal mouse (dotted lines) labeled with anti-D^b mAb. All other K^{b-} mice used in these studies expressed normal levels of D^b. After intravenous transfer, only a fraction of injected cells migrates to the spleen and LN (*27*). The fraction of naïve T cells recovered in female H-$2K^b$–deficient mice corresponds to the fraction of donor cells homing to the pool of lymphoid organs studied (*27*) (Fig. 1).

The fate of memory cells and their level of activation varied markedly in different hosts (Ag^+ right MHC class I > Ag^- right MHC > Ag^- wrong MHC > no MHC) (Fig. 3, C and D). Because all mice received the same cohort of cells simultaneously, these results indicate that the fate of donor cells is conditioned by their interaction with the host environment, and not by interactions with peptides or MHC class I molecules present in the donor cell inoculum. The different kinetics of cell growth also suggests that the affinity of the TCR–MHC peptide interactions may determine a different expansion rate of memory T cells.

The properties of primed $CD8^+$ cells we describe may explain why injection of a virus can sometimes elicit a secondary response to a previously injected but unrelated virus (*2*), mimicking the "original antigenic sin" phenomenon (*31*). They may also explain the kinetics underlying the editing of $CD8^+$ T cell memory. Early after antigen stimulation, the increase in the local production of environment growth factors, as well as the promiscuous stimulation of memory cells, may be responsible for the bystander activation of memory T cells of unrelated specificities (*26*, *32*). The different expansion rate of memory cells, conditioned by the type of TCR-MHC peptide interaction (Fig. 3, C and D), will result in the progressive selection of antigen-specific T cells that will compete out (*33*) T cells bearing unrelated specificities later in the immune response (*34*).

Because our Tg cells express a single TCR, we can exclude nonspecific antigen effects caused by endogenous receptor rearrangements. Thus, in vivo antigen stimulation induced permanent changes in the physiological status of antigen-specific T cells, generating memory cells with unique characteristics. These results are similar to those obtained with cells from normal mice (*11*), so the requirements for survival and expansion may be generalized.

Thus, survival and expansion requirements of $CD8^+$ T cells in the peripheral pools differed for naïve and memory cells and depended on TCR-MHC peptide interactions. Survival of naïve T cells required interactions with H-$2D^b$, similar to thymic positive selection (*15*). Activation and expansion of naïve cells required the presence of the male antigen. In contrast, memory cells required a nonspecific class I interaction for survival and expanded in the presence of the right MHC class I, but in the absence of antigen. Because survival of memory T cells still required interactions with MHC class I, TCR engagement is involved in the survival of memory cells. We do not know what type of peptide recognition is implicated, but cross-reactive peptides may be involved. We cannot exclude, however, promiscuous recognition as in thymic positive selection. Memory T cells may have a lower functional threshold that allows their expansion in the absence of the nominal male peptide, or their survival in the absence of the selecting H-$2D^b$–restricting element. These properties may facilitate both maintenance of memory and secondary immune responses.

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12. Total RNA, used to prepare cDNA, was isolated from sorted (>98% pure) $CD8^+$ T cell populations. $CD8^+$ cells were incubated with anti-CD3 (mAb.1452C11, 1 μg/ml) [O. Leo, M. Foo, D. H. Sachs, L. E. Samelson, J. A. Bluestone, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1374 (1987)] for 6 hours before cell sorting. RNA was reversed-transcribed into cDNA with random oligonucleotides (*35*). All primers used for PCR amplification bridged exon-intron barriers and do not amplify genomic DNA. The primers and probes used for the detection of hypoxanthine-guanine phosphoribosyl-transferase (HPRT), IL-2, IL-4, IL-10, and IFN-γ cDNA have been described (*35*). Other primers and probes were as follows: perforin: 5′-TCTCGCATGTACAGTTTTCGCCTGGTA, 3′-TGTGAGCCCATTCAGGGTCAGCTG (primers), and ATCTCCTCCTATGGCACGCACTTTATCACG (probe); Fas ligand: 5′-CGTGAGTTCACCAACCAAAGC, 3′-CCCAGTTTCGTTGATCACAAG (primers), and GCTCTGATCTCTGGAGTGAAGTATAAGAAA (probe). The 30 cycles of amplification were done as follows: 1 min at 94°C; 1 min at 57°C (53°C for FasL); 1 min at 72°C, followed by 10-min elongation at 72°C. Samples were separated in 1% agarose gels, blotted, and hybridized with ^{35}P-labeled specific probes, and bound radioactivity was analyzed in a PhosphorImager. To ensure semi-quantitative analysis of mRNA production, we selected nonsaturating conditions of amplification.
13. Host mice were irradiated at 600 rayleigh to spare BM precursors, but few peripheral cells were left. Peripheral T cell reconstitution requires previous recolonization of the thymus by host BM precursors, and host T cells start to emerge 1 month after irradiation. Irradiation may prevent the rejection of donor cells. It is also fundamental to allow quantification of T cell decays after adoptive transfer, because donor cells must be initially present at relatively high frequencies, requiring previous depletion of the host's lymphoid organs. In contrast to T cell decays, T cell survival or expansion can be quantified after adoptive transfer into intact mice.
14. M. F. Bachmann, A. Oxenius, T. W. Mak, R. M. Zinkernagel, *J. Immunol.* **155**, 3727 (1995); because class I^- mice do not have $CD8^+$ T cells, we wanted to ensure that decay of Tg cells after transfer into these mice was not due to the recognition and rejection of $CD8^+$ donor Tg cells. Therefore, we used $CD8^-$-deficient mice as class I^+–bearing controls. We found that the recovery of Tg cells after transfer into $CD8^-$ mice was similar to that previously observed after transfer into normal mice (*10*), that is, donor cells were not eliminated and their fate was not modified by the presence of host $CD8^+$ T cells.
15. H-$2D^b$– and H-$2K^b$–deficient mice (B. Pérarnau *et al.*, in preparation); and H-$2D^b$– and β_2M ($D^b \times \beta_2$M)–deficient mice [S. Pascolo *et al.*, *J. Exp. Med.* **185**, 2043 (1997)] obtained by crossing H-$2D^b$–deficient and β_2M-deficient mice [B. H. Koller, P. Marrack, J. W. Kapler, O. Smithies, *Science* **248**, 1227 (1990)] were bred at the Pasteur Institute.
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18. Expression of H-$2D^b$ in β_2M-deficient mice conditions TCR repertoires, because anti-HY TCR Tg β_2M- deficient male mice delete male-specific T cells in the thymus, to an extent similar to that in MHC class I^+ mice [B. Rocha, unpublished data].
19. The absolute number of donor cells present in the host's spleen and a pool of LNs was calculated from the frequency of donor cells and the absolute number of cells present in these organs. To minimize possible TCR-MHC interactions between the cohort of injected T cells (which express class I MHC), we injected relatively low numbers of T cells, which should disperse in host mice. Indeed, interactions between transferred T cells were not sufficient to ensure their survival, as shown by the disappearance of $CD8^+$ T cells in MHC-deficient hosts. To accurately quantify these cells, we analyzed at least 10^5 lymphocytes. In MHC-deficient hosts in which donor cells virtually disappear, we sorted T cells from all LN cell suspensions and half the spleen, to calculate their frequency.
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21. Bromodeoxyuridine (Sigma, 1 mg/mouse) was administered intraperitoneally twice a day for 3 days. Cell populations identified by surface staining were sorted in a FACS-Vintage (Becton Dickinson). Labeling with fluorescein isothiocyanate (FITC)–anti-BrdU mAb (Becton Dickinson) was done as described (*10*).
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23. The very rapid decay of $CD8^+$ T cells in the absence of class I MHC interactions contrasts with the behavior of fetal $CD4^+$ T cells, reported to survive for 6 months in mice lacking class II [S. Takeda, H.-R. Rodewald, H. Arakawa, H. Bluethman, T. Shimizu, *Immunity* **5**, 217 (1996)]. However, fetal and adult T cell populations have such intrinsic differences in kinetic behavior [reviewed in (*36*)] and signal transduction mechanisms that the behavior of fetal cells may not be identical to those of adult T cells.
24. We used this system to immunize Tg cells rather than directly stimulating Tg female mice with male cells to generate homogeneous populations of activated Tg cells. In fact, when female Tg mice are immunized with male cells, a substantial fraction of Tg lymphocytes is never activated by male antigen (B. Rocha, unpublished data).
25. We obtained male→female BM B cell chimeras by injecting irradiated (600 R) 6- to 8-week-old RAG2-deficient female mice with 5×10^6 BM cells (a mixture of 90% Ly5.2 female and 10% Ly5.1 male BM cells) from T cell–deficient CD3ε knockout mice [M. Malissen *et al.*, *EMBO J.* **14**, 4641 (1995)]. These mice had no T cells, and an average of 10% of

BM-derived cells were of male origin, that is, they were Ly5.1. About 5×10^5 naïve cells were injected into male→female chimeras, together with 5×10^5 naïve cells were injected into male→female chimeras, together with 5×10^5 purified $CD4^+$ T cells from B6 female mice. These $CD4^+$ LN cells potentiated the expansion of T cells (in preparation), but elimination of male cells was strictly dependent on the transfer of male-specific Tg cells; it occurs in the absence of $CD4^+$ cells (in preparation) and is not seen after the injection of either $CD4^+$ or $CD8^+$ T cells from non-Tg mice (*8*). Memory cells were recovered from these mice 6 months after T cell transfer (except where noted). These cells were purified by cell sorting or by depletion of B cells, $CD4^+$, and antigen-presenting MHC class II–positive cells by magnetic sorting with coated Dynabeads (Dynal, Byosis, France). For surface staining, we used the following mAbs: biotin-labeled T3.70 (anti-TCRVα Tg) (*15*) (visualized with streptavidin TRICOLOR (Caltag, San Francisco, CA) or streptavidin-allophycocyanin (APC, Molecular Probes, Cambridge, UK); phycoerythrin-labeled mAbs to CD8, B220, and CD44 (Pharmingen, San Diego, CA); Red 16–labeled mAbs to CD8 (Gibco-BRL, UK); and FITC-labeled anti-Ly5.1 (A1042.1) and anti-Thy1.1 (19XE5) [M. W. Lostrom *et al.*, *Virology* **98**, 336 (1979)]. Cell populations were analyzed, and sorted in a FACS-Vintage (Becton Dickinson).

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27. The fraction of memory cells incorporating BrdU in steady-state conditions (20% in male→female chimeras where male antigen was eliminated) is lower than that found when memory cells are transferred into irradiated female class I^+ mice (70%). This difference is to be expected, because it has previously been demonstrated that the rate of cell expansion is dependent on "space" and is increased after adoptive transfer in mice containing few T cells [B. Rocha, N. Dautigny, P. Pereira, *Eur. J. Immunol.* **19**, 905 (1989)].
28. In class I^- mice injected with mixtures of $CD4^+$- and $CD8^+$-bearing cells, donor class I^+ $CD4^+$ T cells did not prevent the decay of $CD8^+$ T cells. This may be because $CD8^+$ T cells require interactions with other types of class I^+ APC to survive. However, the kinetics of $CD8^+$ T cell decay and the kinetics of $CD4^+$ T cell expansion render it very unlikely that, early after transfer, $CD4^+$ T cells would be present in sufficient numbers to interact with $CD8^+$ T cells and prevent their decay. $CD8^+$ T cell decay is very rapid. Injected $CD4^+$ T cell donor cells disperse through the body, and few are found in lymphoid organs early after transfer. At day 3 after injection cells start cycling, but doubling times are relatively slow (48 hours) [B. Rocha, M. P. Lembezat, A. A. Freitas, A. Bandeira, *Eur. J. Immunol.* **19**, 1137 (1989)]. One week after injection we recover 3×10^6 $CD4^+$ T cells in host mice—a small number compared with the total number of class I^+ cells present in a class I^+ mouse.
29. D^b expression in Kb^- mice is highly variable. These mice may be chimeras of D^{b+} and class I^- cells or down-regulate D^b expression to a variable extent. Expression of D^b also varies in different organs (B. Pérarnau *et al.*, in preparation).
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37. In class I–deficient mice, expansion of $CD4^+$ T cells is more marked than in mice expressing class I. This is to be expected, because several studies on the homeostatic regulation of peripheral T cell numbers (*36*) show that $CD4^+$ and $CD8^+$ cells share the same "space." In the absence of $CD8^+$ T cell expansion, $CD4^+$ cells expand more and take over the space of $CD8^+$ cells, and vice versa. At 2 weeks after transfer, the same number of $CD4^+$ cells persist in these hosts.
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