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The clone size of peripheral CD8 T cells is regulated by TCR promiscuity

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Positive selection in the thymus and peripheral T cell survival depend on T cell receptor (TCR)–major histocompatibility complex (MHC) interactions, but it is not yet clear if both events follow exactly the same rules. We studied peripheral T cell survival and clone sizes in conditions of progressive reduction of restricting MHC-bearing cells or progressive ablation of different MHC molecules. Different CD8⁺ T cell clones/polyclonal populations showed different survival and/or lymphopenia-driven proliferation requirements. We could correlate clone sizes to the capacity of each TCR to interact with different types of MHC complexes. Thus, although repertoire selection in the thymus is mainly conditioned by the affinity of TCR–MHC interactions, peripheral selection is determined by TCR cross-reactivity to environmental ligands.

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During the last decade, it has been recognized that the survival of TCR $\alpha\beta$ CD4⁺ and CD8⁺ T cells is an active process depending on TCR signaling (1–7) and on other environmental factors including cytokine receptor-mediated signals (8, 9). The relative contribution of these environmental clues to homeostasis, T cell survival, and lymphopenia-driven division is not yet fully understood.

The role of TCR signaling was initially studied by transferring monoclonal CD8 T cell populations to MHC class I-deficient mice. In these circumstances, T cell survival varied. Naive, anti-HY transgenic (Tg) monoclonal T cells disappeared rapidly in the absence of H-2D^b (1), whereas the decay of H-2D^b-restricted P14 T cells was slower (4). Further differences were reported when memory CD8⁺ T cell populations were studied in similar contexts. Survival of aHY CD8⁺ memory T cells did not require interactions with H-2D^b, demonstrating that TCR-mediated survival signals could vary during the life-history/differentiation stage of the same mature T cell clone (1). However, aHY cells did not survive in β_2m and H-2D^b-deficient hosts (1). In contrast, lymphocytic choriomeningitis virus-specific CD8⁺ memory T cells survived in this defi-

cient background (4), suggesting heterogeneity in CD8 T cell behavior.

Studies of conditional ablation of the TCR supported this notion. Polyclonal CD8⁺ T cells take up to 1 mo to disappear (6, 7), contrasting with their faster decay observed after T cell transfer into MHC-deficient hosts (1). The slow progressive decay of the TCR-deficient T cells could be caused by the slow purging of surface TCR after gene ablation (7), which could allow T cells to adapt by changing survival signaling thresholds (10, 11). In contrast, upon transfer into MHC-deficient hosts, donor T cells are confronted with an acute lack of recall elements and would not have the time to undergo any adaptation process (10, 11). These differences could also be caused by intrinsic caveats of the experimental systems used. Adoptive T cell transfer into MHC-deficient hosts required previous irradiation (600–700 rads) to prevent donor T cell elimination by the hosts T cells. Irradiation changes the cytokine environment, thus creating conditions that are not present in normal mice. Upon TCR ablation it cannot be excluded that the presence of TCR at the cell membrane might only be required to provide constitutive signals necessary for T cell survival (7). In these circumstances, cell decay could be caused by the mere lack of the T cell receptor, and not by the absence of signals induced by TCR–MHC interactions. In spite of these caveats, both approaches highlighted the possibility that different clones could have different survival requirements.

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The online version of this article contains supplemental material.

Previous studies have correlated the expansion capacity of different T cell clones to TCR density and CD5 expression, suggesting that TCR affinity determines peripheral survival and lymphopenia-driven proliferation (LDP) (12, 13). It is, however, not clear from these studies which properties determine the observed LDP differences: if they were caused by intrinsic TCR properties such as TCR affinity or rather TCR density, a broader TCR cross-reactivity, or even to the different capacity of each clone to react to other environmental clues, such as cytokines. In the present investigation, we developed new experimental approaches that could overcome some of the caveats of previous systems and used these to identify the survival requirements of different CD8⁺ TCR-Tg clones in vivo.

RESULTS AND DISCUSSION

To correlate individual TCRs to homeostasis of pool sizes, we used B6 Rag-deficient mice, expressing Tg TCRs restricted to either MHC class I H-2D^b (aHY, P14) or H-2K^b (OT-1). We initially studied the characteristics of the peripheral T cell pool in intact mice in the absence of either adoptive cell transfer or LDP. The total number of peripheral CD8⁺ T cells varied markedly. It was lower in aHY ($\sim 5\text{--}6 \times 10^6$), doubled in P14 ($\sim 13 \times 10^6$), and three- to fourfold higher ($\sim 20 \times 10^6$) in OT-1 mice. These different pool sizes correlated with the “activation state” of the T cells (Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20052174>). Indeed, T cell activation induces TCR and CD8 down-regulation and the up-regulation of negative regulator of TCR signaling CD5 and of the CD44 marker. The TCR and CD8 levels were higher in aHY, intermediate in P14, and lower in OT-1 cells. In contrast, the expression of both CD5 and CD44 followed the opposite trend, i.e., they were higher in OT-1, intermediate in P14, and lower in aHY cells. Thus, the T cell activation status correlated directly to T cell pool sizes and followed the order OT1>P14>HY. Since all these mice lacked the nominal antigen recognized by their Tg TCR, the different activation state and pool sizes should result from other interactions with their environment. The availability of several TCR-Tg strains with potentially different reactivity to MHC/environmental antigens allows us to address the impact of these interactions on cell survival, LDP, and niche replenishment. We therefore compared the behavior of these different clones in conditions where the interactions between their TCR and the environmental MHC-peptide complexes were limiting.

We first studied the consequences of ablation of selective MHC class I molecules. Survival of the H-2D^b-restricted aHY and P14 cells was strictly dependent on TCR-H-2D^b interactions. When transferred to female CD3 ϵ ^{-/-}H-2D^b^{-/-} aHY Tg cells died faster than P14 cells, but in both cases most cells had disappeared at 24 h and none was detected 2 d after transfer (Fig. 1, A and B). The selective death of T cells in H-2D^b^{-/-} hosts was not caused by either defective homing or cell rejection because the only cells dying in H-2D^b^{-/-} hosts were the H-2D^b-restricted T cells since polyclonal T cells

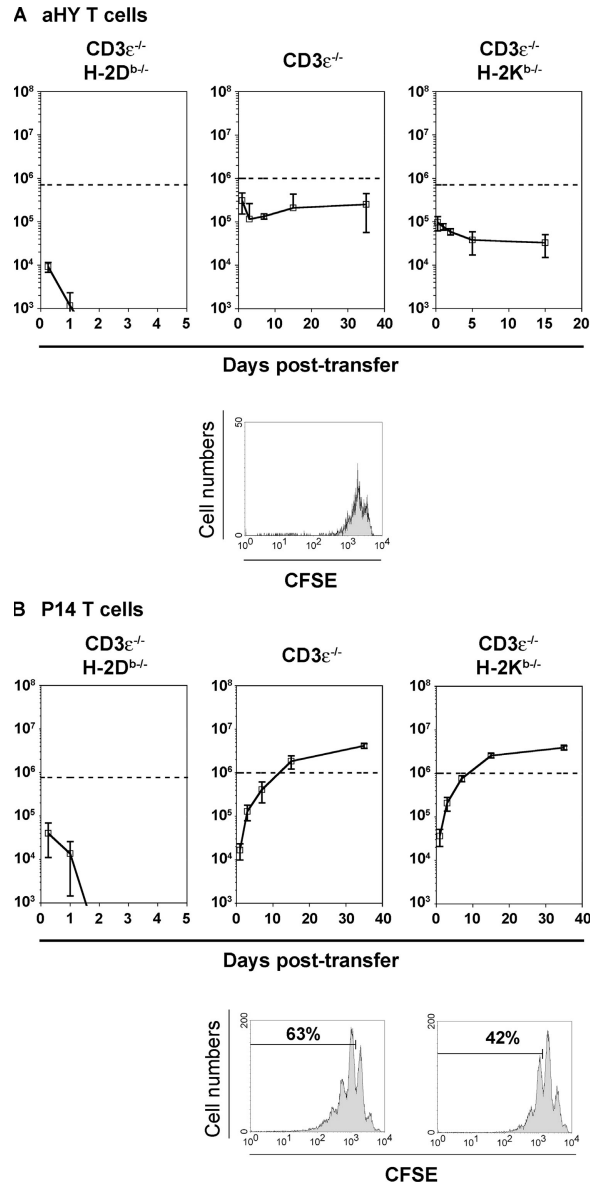
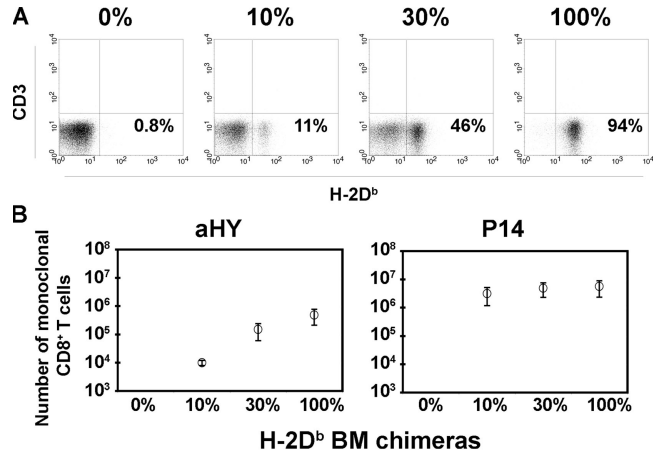


Figure 1. Kinetics of aHY and P14 T cell fate after transfer.

(A) Between 0.8 and 1.2×10^6 LN cells from monoclonal aHY mice were transferred into B6.CD3 ϵ ^{-/-}H-2D^b^{-/-} (top left), B6.CD3 ϵ ^{-/-} (top middle), or B6.CD3 ϵ ^{-/-}H-2K^b^{-/-} (top right) mice. The bottom panel shows the CFSE dilution patterns 7 d after transfer in B6.CD3 ϵ ^{-/-} hosts. (B) LN cells from monoclonal P14 mice were transferred into B6.CD3 ϵ ^{-/-}H-2D^b^{-/-} (top left), B6.CD3 ϵ ^{-/-} (top middle), or B6.CD3 ϵ ^{-/-}H-2K^b^{-/-} (top right) mice (0.8 and 10^6 , respectively). The bottom panels show the CFSE dilution patterns 7 d after transfer in B6.CD3 ϵ ^{-/-} (left) and B6.CD3 ϵ ^{-/-}H-2K^b^{-/-} (right) hosts. The fraction of cells that divided two or more times is shown. The dashed line represents the number of injected cells. Results shown correspond to the number of donor CD8⁺ T cells recovered from spleen and LN of the three to five host mice (mean \pm SD). Similar results were obtained in two to four independent experiments. Long-term recovery of donor cells was studied in sublethally irradiated host mice (450 rads).

survived well (Fig. S2, available at <http://www.jem.org/cgi/content/full/jem.20052174>). The behavior of the aHY and P14 T cells differed upon transfer into CD3 $\epsilon^{-/-}$ and CD3 $\epsilon^{-/-}$ H-2K $^{b/-/-}$ female hosts (Fig. 1, A and B). The aHY cells survived at relative constant numbers in H-2D $^{b+}$ hosts, whereas P14 CD8 $^{+}$ T cells proliferated to reach a plateau around $2-4 \times 10^6$ cells. These latter findings indicate that survival and LDP are closely related and that to expand P14 cells must first receive survival signals. We were surprised, however, to observe that P14 divided more in CD3 $\epsilon^{-/-}$ mice than in CD3 $\epsilon^{-/-}$ H-2K $^{b/-/-}$ mice (Fig. 1 B). Since these hosts differed only by the presence of H-2K b in CD3 $\epsilon^{-/-}$ mice, these results indicated that P14 cells (although H-2D b restricted) could also interact with H-2K b -peptide complexes. In these circumstances, they raise the possibility that TCR cross-reactivity may contribute to T cell behavior in the peripheral pools.

Since HY and P14 CD8 $^{+}$ T cells interacting with H-2D b MHC class I molecules have different LPD and the P14 TCR may cross-react with H-2K b , we studied if their survival requirements overlapped in limiting conditions of H-2D b availability. We generated hosts containing different numbers of H-2D $^{b+}$ cells by injecting irradiated CD3 $\epsilon^{-/-}$ $\beta_2m^{-/-}$ H-2D $^{b/-/-}$ H-2K $^{b/-/-}$ mice (tetra KO) with BM cells from CD3 $\epsilon^{-/-}$ H-2D $^{b/-/-}$ donors alone or containing 10, 30, or 100% CD3 $\epsilon^{-/-}$ H-2D $^{b+}$ BM cells. After reconstitution, the H-2D $^{b+}$ cell representation in the chimeras was proportional to the fraction of H-2D $^{b+}$ BM cells injected (Fig. 2 A). We transferred equal numbers of aHY and P14 CD8 $^{+}$ T cells into these chimeras and studied Tg cell recovery 1 mo later. The survival of aHY cells was acutely sensitive to any reduction in H-2D b availability; cell yields were reduced 10-fold in 30% H-2D $^{b+}$ chimeras and 100-fold in 10% H-2D $^{b+}$ chimeras (Fig. 2 B). In contrast, the yield of P14 Tg cells was comparable in mice containing 100 or 30% of H-2D $^{b+}$ cells and only two- to threefold reduced in chimeras with 10% H-2D $^{b+}$ BM-derived cells (Fig. 2 B). As expected, both aHY and P14 cells disappeared in chimeras without H-2D b -bearing cells (Fig. 2 B). These experiments demonstrated that the number of MHC class I-expressing cells affects cell survival and that T cells bearing different TCRs fare differently when the frequency of T cell encounters with the “correct” APCs decreases. In these conditions, long term T cell survival indicates that either T cells remain attached to H-2D $^{b+}$ APCs—cell contact is permanent—or in agreement to the “serial encounter model”(14) T cell survival and/or LDP is possible when the interaction is intermittent. It is currently assumed that survival and LDP are conditioned by the affinity of the interaction between the Tg TCR and MHC-expressing cells. As P14 cells survive better H-2D b depletion than aHY cells, the “affinity” of interaction of the P14 TCR with the H-2D $^{b+}$ APCs should be higher. We addressed this question directly by studying competition between aHY and P14 cells in conditions of reduced H-2D b availability. In these conditions cells expressing a TCR with a higher affinity for H-2D b -peptide complexes should out-compete cells with a low-affinity



H-2D b BM chimeras	MoaHY		MoP14	
	Alone	With P14	Alone	With aHY
30%	0.15 \pm 0.09	0.05 \pm 0.04	4.9 \pm 2.5	1.1 \pm 0.4
100%	0.49 \pm 0.28	0.20 \pm 0.06	5.7 \pm 3.3	2.7 \pm 2.1

Figure 2. T cell fate in the presence of reduced amounts of MHC class I-expressing cells. (A) BM chimeras containing limited numbers of H-2D b MHC class I-expressing cells were produced by reconstituting CD3 $\epsilon^{-/-}$ $\beta_2m^{-/-}$ H-2D $^{b/-/-}$ H-2K $^{b/-/-}$ tetra KO mice with mixtures of BM cells from CD3 $\epsilon^{-/-}$ H-2D $^{b+}$ (0, 10, 30, and 100%) and CD3 $\epsilon^{-/-}$ H-2D $^{b/-/-}$ mice. Plots show representative proportions of H-2D $^{b+}$ hematopoietic cells in the BM chimeras of the four experimental groups. (B) 5 wk after reconstitution, 10^6 LN cells from aHY or P14 mice were transferred into the BM chimeras described in A. Results show the number of donor T cells recovered 4 wk after T cell transfer in five to seven different BM chimeras. Similar results were obtained in three independent experiments. (C) BM chimeras containing either 30 or 100% of H-2D $^{b+}$ hematopoietic cells received 10^6 aHY or/and P14 CD8 $^{+}$ T cells injected alone or coinjected. Mice were killed 4 wk later, and the number of aHY and P14 CD8 $^{+}$ T cells was assessed. Values show the mean \pm SE of the number ($\times 10^6$) of T cells recovered in the spleen and LNs of four to seven mice. MoaHY, monoclonal aHY; MoP14, monoclonal P14.

TCR. We found that the presence of P14 cells considerably reduces aHY cell recovery (Fig. 2 C). Competition was more marked in chimeras with only 30% D $^{b+}$ BM-derived cells (threefold reduction) than in chimeras containing 100% D $^{b+}$ BM (twofold reduction). Surprisingly, we found that the aHY Tg cells appeared more efficient competitors than P14 Tg cells. Thus, although the P14 cells undergo LDP and reach higher numbers, the less abundant aHY cells were still able to prevent the survival of a substantial fraction of P14 cells. These results suggest aHY and P14 cells do not compete for cytokines, but for interactions with H-2D b as it only occurs when D b is limiting. They also suggest that the larger clone size of the P14 cells may not be determined by their

higher TCR affinity. Moreover, other results actually suggest that the aHY TCR may even have a higher affinity to MHC than the P14 receptor, because aHY out-compete P14 cells for positive selection in the thymus (15).

It is likely that each TCR cross-reacts and recognizes a broad spectrum of different H-2D^b-peptide complexes with a wide range of affinities. The overall assembly of recognized complexes defines niche size. The patterns of recognition by two different TCRs may partially overlap. In this case the higher affinity of one population for some complexes will allow it to prevail and out compete a second population and vice-versa, i.e., a low affinity for a different set of complexes will determine its disappearance in presence of competitors. Adoptive cell transfer experiments suggested that T cell populations occupy precise niches according to the TCR fine specificity (16, 17). We suggest that niche occupancy during T cell survival/LDP is regulated by competition for ligands that may partially overlap (18).

The H-2K^b-restricted OT-1 CD8⁺ naive Tg cells behaved differently from both aHY and P14 Tg cells. They proliferated extensively in mice expressing K^b (Fig. 3 A) to reach a plateau at ~8–10 × 10⁶ cells (average of 10 experiments), 2–3 fold higher than P14 cells. Moreover, in the absence of K^b, OT-1 T cells did not perish but in fact proliferated. The absence of K^b delayed cell proliferation and

accumulation and reduced plateau level to 4–5 × 10⁶ cells (Fig. 3, A and B). Therefore, most OT-1 cells did not require MHC class I-restricted interactions for peripheral survival/LDP. This observation appeared to contradict previous data indicating that naive T cell survival and LDP is dependent on TCR–MHC interactions. Alternatively, OT-1 T cells could interact with different MHCs besides H-2K^b. To investigate this possibility, we studied the survival of OT-1 CD8⁺ T cells in CD3ε^{-/-}β2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-} tetra KO hosts, which should express very low levels of MHC class I. We found that removal of additional MHC class I molecules induced a major delay in OT-1 cell accumulation. Cell recovery was at any time point reduced up to 10-fold compared with K^b-expressing hosts, but yet some OT-1 CD8⁺ T cells were able to persist (Fig. 3 A). These results confirmed that the OT-1 TCR cross recognize multiple MHC class I molecules. Interestingly, upon transfer of the OT-1 T cells in tetra KO mice, we observed the emergence of a population of CD4 T cells expressing the OT-1 TCR (Fig. 3 C), which represented less than 1% of the injected population, suggesting that the OT-1 TCR may recognize MHC class II-peptide complexes (Fig. S3, available at <http://www.jem.org/cgi/content/full/jem.20052174>). To evaluate if the residual survival of OT-1 cells in MHC class I-deficient mice was caused by MHC class II recognition,

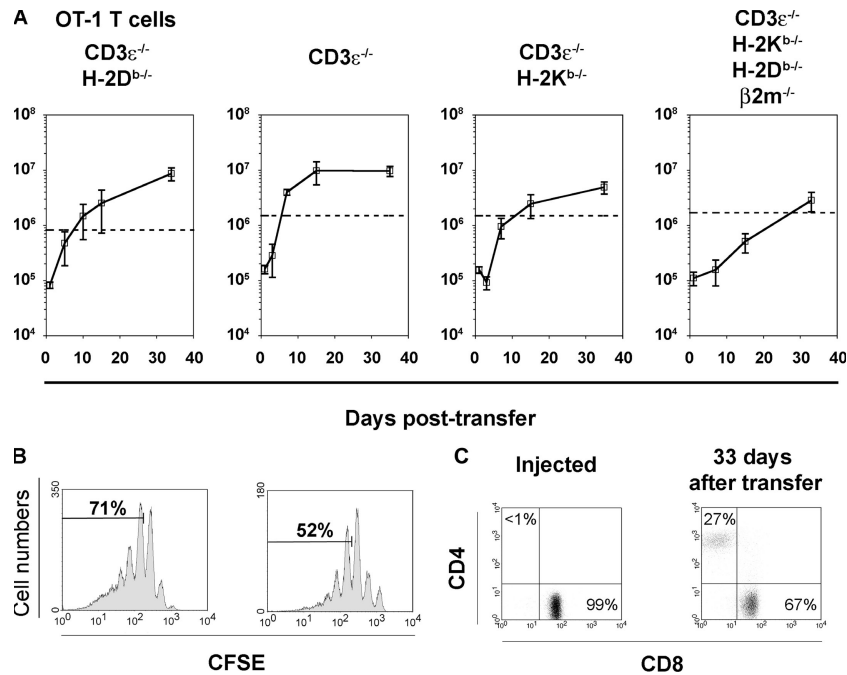


Figure 3. Kinetics of OT-1 T cell fate after transfer. LN cells from monoclonal OT-1 mice (0.9–1.5 × 10⁶) were transferred into different sublethally irradiated host mice (450 rads). Results shown correspond to the number of donor CD3⁺CD8⁺ T cells recovered from spleen and LN of three to five different host mice at different times after transfer (mean ± SD) (when SD bars are not seen they were smaller than the symbol). Similar results were obtained in two to five independent experiments. The dashed line represents the number of injected cells.

(A) OT-1 cell fate in CD3ε^{-/-}H-2D^b^{-/-}, CD3ε^{-/-}, CD3ε^{-/-}H-2K^b^{-/-} mice and CD3ε^{-/-}β2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-} tetra KO mice. (B) CFSE dilution patterns obtained 7 d after transfer in CD3ε^{-/-} (left) and CD3ε^{-/-}H-2K^b^{-/-} (right) hosts. The fraction of donor cells that divided three or more times is shown. (C) Dot plots show the fractions of gated Tg TCR⁺ (Vβ5⁺Vα2⁺) OT-1 CD4 and CD8 T cells among the injected cells and in the LN of the CD3ε^{-/-}β2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-} tetra KO recipient mice 33 d later.

we transferred OT-1 cells into $\beta 2m^{-/-}I-A\beta^{-/-}$ hosts. Ablation of MHC class II further reduced OT-1 cell proliferation and recovery (Fig. 4). We failed to recover CD8⁺ donor T cells in one out of three $\beta 2m^{-/-}I-A\beta^{-/-}$ mice, and CD8⁺ T cell numbers in two other mice were significantly less than in $CD3\epsilon^{-/-}\beta 2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-}$ mice. Thus, the number of OT-1 cells recovered was strongly influenced by the removal of each type of MHC molecules in the host mice. Compared with K^b-expressing hosts, peripheral survival/LDP of OT-1 cells is hindered by H-2K^b deficiency to 2-fold lower levels, further reduced to over 5-fold lower levels in $CD3\epsilon^{-/-}\beta 2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-}$ mice (Fig. 3 A), and even more reduced to 10-fold lower values and some times totally abrogated in $\beta 2m^{-/-}I-A\beta^{-/-}$ hosts (Fig. 4). Thus, even when cytokine accessibility was not limiting T cell proliferation was proportional to MHC availability. These findings suggest that cross-reactive TCR-MHC interactions play a determinant role in the initial drive to the differential survival and expansion of CD8⁺ T cells. It has

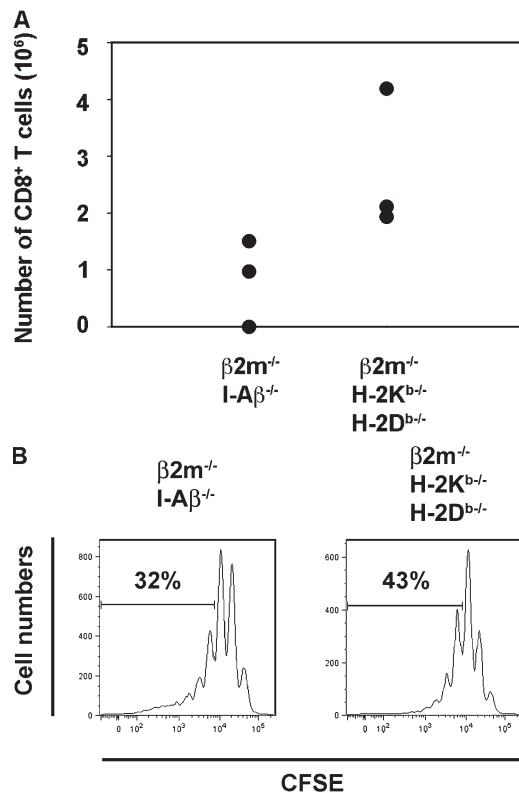


Figure 4. Fate of OT-1 cells in MHC class II-deficient mice. (A) About 10^6 LN OT-1 CD8⁺ T cells were transferred into sublethally irradiated (450 rads) $Rag2^{-/-}\gamma c^{-/-}\beta 2m^{-/-}I-A\beta^{-/-}$ (left) and $CD3\epsilon^{-/-}\beta 2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-}$ tetra KO mice (right). Results show the number of CD8⁺ donor T cells recovered from spleen and LN of individual host mice 30 d after transfer. Cell recovery was similarly decreased in both spleen and LNs of the $I-A\beta^{-/-}$ mice. (B) The bottom panels show the CFSE dilution 7 d after transfer in B6. $I-A\beta^{-/-}$ (left) $CD3\epsilon^{-/-}\beta 2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-}$ (middle) and B6. $\beta 2m^{-/-}I-A\beta^{-/-}$ (right) hosts. The fraction of donor cells that divided three or more times is shown.

been claimed that activated/memory T cells are no longer concerned about MHC or TCR signals and that their survival or expansion is strictly dependent on cytokine signals (8, 9, 19). However, conditional TCR ablation leads to the disappearance of both naive and memory cells (6, 7). Thus, it is likely that survival of memory CD8⁺ T cells still requires TCR-mediated recognition.

A population of polyclonal CD8⁺ T cells is likely to contain individual clones with the survival requirements of either the aHY or the OT-1 clones. Previous reports have suggested that memory T cell clones may differ in their survival requirements (1, 4). We thus evaluated the fate of polyclonal naive CD44⁻ and activated/memory CD44^{high} CD8⁺ T cells after transfer into MHC class I-deficient tetra KO mice (Fig. 5). We found that naive cell recovery at day 3 was 10-fold lower than in MHC intact hosts (1% compared with 10% of the injected cells in $CD3\epsilon^{-/-}$ hosts), suggesting that most donor cells are lost early after transfer. The remaining naive CD44⁻CD8⁺ T cells expanded thereafter with slow kinetics and large individual variations. The number of cells recovered was 100-fold lower than in MHC-expressing hosts. Activated CD44^{high}CD8⁺ cells also underwent a sharp initial decay, similar to naive cells, excluding the role of cytokine dependency in the initial cell survival. The remaining activated/memory cells, however, expanded faster and reached 10-fold higher numbers compared with naive cells. Total CD8⁺ cell recovery (less than 10^6), however, never reached the plateau obtained in $CD3\epsilon^{-/-}$ MHC class I⁺ hosts (Fig. 5). Thus, although the majority of activated/memory cells fail to survive early after transfer into MHC class I-deficient hosts, the surviving cells proliferate better than their naive counterparts, likely caused by their increased responsiveness

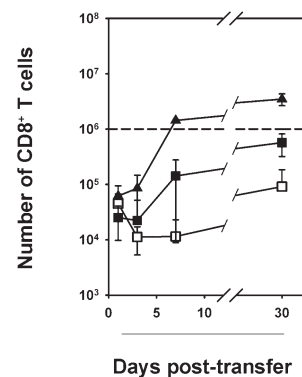


Figure 5. Polyclonal CD8⁺ T cell transfers in MHC class I-deficient mice. 10^6 sorted CD44⁻ (□) and CD44^{high} (■) CD8⁺ LN T cells from B6 mice were transferred into sublethally irradiated (450 rads) $CD3\epsilon^{-/-}\beta 2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-}$ tetra KO mice. The dashed line represents the number of injected cells. Kinetics of CD8 cell recovery in $CD3\epsilon^{-/-}$ MHC class I⁺ hosts is also shown (▲). Results shown correspond to the number of CD8⁺ T cells recovered from spleen and LN of the three to six host mice (mean \pm SD) at different times after transfer. CD4⁺ T cells reached a plateau of more than 10^7 cells in both $CD3\epsilon^{-/-}$ and tetra KO mice.

to homeostatic cytokines (18). It remains to be explained why only a minor fraction of the memory cells undergo expansion. A likely explanation is that such cells have increased cross-reactivity as we found to be the case of OT-1 cells. It should be pointed out that these observations do not exclude the role of cytokines. They suggest that T cells require initial TCR tickling to survive before undergoing cytokine-dependent responses. TCR-mediated activation by either cross-reactive or specific ligand recognition may change T cell responsiveness to cytokines (19) and contribute to the establishment of independent homeostatic controls for naive and memory CD8⁺ T cells (20).

T cell selection in the thymus has been shown to be dependent on the affinity of TCR interactions with self-MHC. It was therefore assumed that similar factors would determine peripheral T cell survival (1). Individual T cell clones are known to differ in survival and LDP requirements, and it was postulated that these differences were caused by different TCR affinities for self-ligands (12, 13). By studying T cell numbers in intact monoclonal mice and by comparing survival/LDP requirements and clonal competition in situations where only the interactions between their TCR and MHC-peptide complexes were limiting, we show that clone sizes in the periphery are determined by the number of MHC-peptide complexes, as well by the capacity of each TCR to interact with multiple MHC types. These properties, which determine clone size, may also determine peripheral selection of T cell repertoires. Cells with promiscuous TCR are preferentially selected into the activated/memory pool (21). Thus, in contrast to T cell positive selection in the thymus that is mainly conditioned by TCR affinity, peripheral clones sizes appear to be determined by TCR promiscuity.

MATERIALS AND METHODS

Mice. C57BL/6 (B6) Ly5^a and Ly5^b mice were from Charles River/Iffa-Credo. TCR Tg monoclonal mice in a B6.Rag2^{-/-} genetic background were: monoclonal aHY bearing a V α T3.70.V β 8.2 H-2D^b-restricted TCR Tg specific for the HY male antigen and monoclonal P14 bearing a V α 2.V β 8.1, H-2D^b-restricted TCR Tg specific for the gp33-41 epitope of the lymphocytic choriomeningitis virus glycoprotein. Monoclonal OT-1 mice with a V α 2.V β 5 H-2K^b-restricted TCR Tg specific for the 257-264 peptide of ovalbumin were B6.Rag1^{-/-}. Mice lacking H-2D^b or H-2K^b (22) were crossed with T cell-deficient B6.CD3 ϵ ^{-/-} mice. Tetra knockout mice lacking classic MHC class I molecules (CD3 ϵ ^{-/-} β 2m^{-/-}H-2D^b^{-/-}H-2K^b^{-/-}) were made. The T cell-deficient CD3 ϵ ^{-/-} mice and the B6. β 2m^{-/-} mice were from the Centre de Distribution, Typage & Archivage animal (CDTA). B6.Rag2^{-/-} γ c^{-/-} β 2m^{-/-}I-A β ^{-/-}, which lack MHC class II were from O. Lantz (Institute Curie, Paris, France). All mutant mouse strains backcrossed into the B6 background were kept at the CDTA or in our SPF animal facilities according to the Experimental Ethics Committee guidelines.

Mice deficient in MHC class I H-2D^b, H-2K^b, or β 2m were backcrossed into the C57BL/6 background and further crossed with C57BL/6 CD3 ϵ -deficient mice. Thus the CD3 ϵ and MHC class I-deficient mice generated have major advantages when compared to MHC-deficient CD3 ϵ ⁺ counterparts. Since they cannot generate endogenous T cells able to reject transferred MHC class I-bearing T cells, they do not require strong irradiation before cell transfer and therefore allow the long-term follow-up of the donors cells. However, we found that although in H-2K^b^{-/-} or tetra KO, but not in H-2D^b^{-/-} mice, donor T cells decay 2 wk after transfer (not

shown). This decay was not modified by anti-NK1.1 treatment (not shown) or anti-CD16/23 treatment (not shown), but was abrogated by sub-lethal irradiation (300–450 rads) of the hosts.

Adoptive T cell transfer and CFSE staining. LNs of monoclonal TCR Tg mice were used as a source of homogeneous CD8⁺ T cells. Polyclonal LN CD8⁺ or CD4⁺ T cells from wild-type B6 mice were purified using magnetic cell sorting (MACS; Milteny Biotech). Purified T cells ($\pm 10^6$) were injected i.v. into T cell-deficient hosts, lacking or not lacking MHC class I molecules. By using mice with different Ly5 allotypes and transgenic TCR chains, we were able to discriminate T cells from different donors. To study long-term T cell persistence hosts were sublethally irradiated (450 rads) before cell transfer. Hosts were killed at different times after transfer. Spleen, inguinal, and mesenteric LNs cells were prepared, and the number and phenotype of donor CD3⁺CD8⁺ (and in one case CD3⁺CD4⁺; see Results and discussion) T cells evaluated. T cell division after transfer was studied by dilution of CFSE. To compare the rate of cell division of the transferred cells we choose to show the fraction of donor cells that divided more than two or three times, 7 d after transfer.

BM chimeras. BM chimeras were made by lethal irradiation of host mice followed by BM transfusion. We generated mice containing variable numbers of BM-derived cells expressing MHC class I. Tetra KO mice were lethally irradiated (900 rads) with a ¹³⁷Ce source and rescued by the i.v. injection of 5×10^6 BM cells. The BM inoculums contained cells from different MHC class I⁺ and class I⁻ donors mixed at variable ratios.

Flow cytometry. Spleen and LN cells were stained with appropriate combination of different monoclonal antibodies. APC- (Caltag) or PerCP-coupled (Becton Dickinson) streptavidin was used as a second step reagent. Dead cells were excluded according to their light-scattering characteristics. All acquisitions were done with a FACScalibur (Becton Dickinson) interfaced to the Macintosh CellQuest software.

Online supplemental material. Fig. S1 shows the phenotypic characterization of the different monoclonal CD8 T cells used in this study. Fig. S2 shows and discusses the fate of polyclonal CD4 and CD8 T cells when transferred into different CD3 ϵ ^{-/-} MHC class I-deficient hosts. Fig. S3 shows results obtained in MHC class I-deficient chimeras reconstituted with BM cells from OT-1 donors. Figs. S1–S3 are available at <http://www.jem.org/cgi/content/full/jem.20052174>.

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REFERENCES

1. Tanchot, C., F.A. Lemonnier, B. Perarnau, A.A. Freitas, and B. Rocha. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science*. 276:2057–2062.
2. Brocker, T. 1997. Survival of mature CD4 T lymphocytes is dependent on major histocompatibility complex class II-expressing dendritic cells. *J. Exp. Med.* 186:1223–1232.
3. Kirberg, J., A. Berns, and H. von Boehmer. 1997. Peripheral T cell survival requires continual ligation of the T cell receptor to major histocompatibility complex-encoded molecules. *J. Exp. Med.* 186: 1269–1275.

4. Murali-Krishna, K., L.L. Lau, S. Sambhara, F. Lemonnier, J. Altman, and R. Ahmed. 1999. Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science*. 286:1377–1381.
5. Witherden, D., N. van Oers, C. Waltzinger, A. Weiss, C. Benoist, and D. Mathis. 2000. Tetracycline-controllable selection of CD4(+) T cells: half-life and survival signals in the absence of major histocompatibility complex class II molecules. *J. Exp. Med.* 191:355–364.
6. Labrecque, N., L.S. Whitfield, R. Obst, C. Waltzinger, C. Benoist, and D. Mathis. 2001. How much TCR does a T cell need? *Immunity*. 15:71–82.
7. Polic, B., D. Kunkel, A. Scheffold, and K. Rajewsky. 2001. How alpha beta T cells deal with induced TCR alpha ablation. *Proc. Natl. Acad. Sci. USA*. 98:8744–8749.
8. Jameson, S.C. 2002. Maintaining the norm: T-cell homeostasis. *Nat. Rev. Immunol.* 2:547–556.
9. Seddon, B., P. Tomlinson, and R. Zamoyska. 2003. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. *Nat. Immunol.* 4:680–686.
10. Tanchot, C., D.L. Barber, L. Chiodetti, and R.H. Schwartz. 2001. Adaptive tolerance of CD4+ T cells in vivo: multiple thresholds in response to a constant level of antigen presentation. *J. Immunol.* 167:2030–2039.
11. Legrand, N., and A.A. Freitas. 2001. CD8+ T lymphocytes in double alpha beta TCR transgenic mice. I. TCR expression and thymus selection in the absence or in the presence of self-antigen. *J. Immunol.* 167:6150–6157.
12. Kassiotis, G., R. Zamoyska, and B. Stockinger. 2003. Involvement of avidity for major histocompatibility complex in homeostasis of naive and memory T cells. *J. Exp. Med.* 197:1007–1016.
13. Kieper, W.C., J.T. Burghardt, and C.D. Surh. 2004. A role for TCR affinity in regulating naive T cell homeostasis. *J. Immunol.* 172:40–44.
14. Friedl, P., and M. Gunzer. 2001. Interaction of T cells with APCs: the serial encounter model. *Trends Immunol.* 22:187–191.
15. Freitas, A.A., F. Agenes, and G.C. Coutinho. 1996. Cellular competition modulates the survival and selection of CD8 T cells. *Eur. J. Immunol.* 26:2640–2649.
16. Moses, C.T., K.M. Thorstenson, S.C. Jameson, and A. Khoruts. 2003. Competition for self ligands restrains homeostatic proliferation of naive CD4 T cells. *Proc. Natl. Acad. Sci. USA*. 100:1185–1190.
17. Troy, A.E., and H. Shen. 2003. Cutting edge: homeostatic proliferation of peripheral T lymphocytes is regulated by clonal competition. *J. Immunol.* 170:672–676.
18. Freitas, A.A., and B. Rocha. 2000. Lymphocyte population biology: the flight for survival. *Annu. Rev. Immunol.* 18:83–111.
19. Schluns, K.S., and L. Lefrancois. 2003. Cytokine control of memory T-cell development and survival. *Nat. Rev. Immunol.* 3:269–279.
20. Tanchot, C., and B. Rocha. 1995. The peripheral T cell repertoire: independent homeostatic regulation of virgin and activated CD8+ T cell pools. *Eur. J. Immunol.* 25:2127–2136.
21. Selin, L.K., and R.M. Welsh. 2004. Plasticity of T cell memory responses to viruses. *Immunity*. 20:5–16.
22. Pascolo, S., N. Bervas, J.M. Ure, A.G. Smith, F.A. Lemonnier, and B. Perarnau. 1997. HLA-A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice. *J. Exp. Med.* 185:2043–2051.