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During the last decade, it has been recognized that the survival of TCRαβ 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-2Db (1), whereas the decay of H-2Dd–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-2Db, 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 β2m and H-2Dd–deficient hosts (1). In contrast, lymphocytic choriomeningitis virus–specific CD8+ memory T cells survived in this deficient 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.
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-2Db (aHY, P14) or H-2Kb (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 (~5–6 x 10^6), doubled in P14 (~13 x 10^6), and three- to fourfold higher (~20 x 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>aHY. 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-2Db-restricted aHY and P14 cells was strictly dependent on TCR–H-2Db interactions. When transferred to female CD3ε^-/-H-2Db^-/- 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-2Db^-/- hosts was not caused by either defective homing or cell rejection because the only cells dying in H-2Db^-/- hosts were the H-2Db-restricted T cells since polyclonal T cells...
survived well (Fig. S2, available at available at http://www.jem.org/cgi/content/full/jem.20052174). The behavior of the aHY and P14 T cells differed upon transfer into CD3ε−/− and CD3ε−/−H-2Kb−/− female hosts (Fig. 1, A and B). The aHY cells survived at relative constant numbers in H-2Db+ hosts, whereas P14 CD8+ T cells proliferated to reach a plateau around 2–4 × 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 CD3ε−/− mice than in CD3ε−/−H-2Kb−/− mice (Fig. 1 B). Since these hosts differed only by the presence of H-2Kb in CD3ε−/− mice, these results indicate that P14 cells (although H-2Db restricted) could also interact with H-2Kb–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-2Db MHc class I molecules have different LPD and the P14 TCR may cross-react with H-2Kb, we studied if their survival requirements overlapped in limiting conditions of H-2Db availability. We generated hosts containing different numbers of H-2Db+ cells by injecting irradiated CD3ε−/−B2m−/−H-2Db+ε−/−H-2Kb+ε−/− mice (tetra KO) with BM cells from CD3ε−/−H-2Db−/− donors alone or containing 10, 30, or 100% CD3ε−/−H-2Db+ BM cells. After reconstitution, the H-2Db+ cell presentation in the chimera was proportional to the fraction of H-2Db+ BM cells injected (Fig. 2 A). We transferred equal numbers of aHY and P14 CD8+ T cells into these chimeras and studied T cell recovery 1 mo later. The survival of aHY cells was acutely sensitive to any reduction in H-2Db availability; cell yields were reduced 10-fold in 30% H-2Db+ chimeras and 100-fold in 10% H-2Db+ chimeras (Fig. 2 B). In contrast, the yield of P14 T cells was comparable in mice containing 100 or 30% of H-2Db+ cells and only two- to threefold reduced in chimeras with 10% H-2Db+ BM-derived cells (Fig. 2 B). As expected, both aHY and P14 cells disappeared in chimeras without H-2Db-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-2Db+ APCs—cell contact is permanent—or in agreement to the “serial encounter model”(14) T cell survival and/or LPD is possible when the interaction is intermittent. It is currently assumed that survival and LPD are conditioned by the affinity of the interaction between the Tg TCR and MHC-expressing cells. As P14 cells survive better H-2Db depletion than aHY cells, the “affinity” of interaction of the P14 TCR with the H-2Db+ APCs should be higher. We addressed this question directly by studying competition between aHY and P14 cells in conditions of reduced H-2Db availability. In these conditions expressing a TCR with a higher affinity for H-2Db–peptide complexes should out-compete cells with a low–affinity 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+ BM-derived cells (threefold reduction) than in chimeras containing 100% D+ 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 LPD 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 H2-D+ as it only occurs when D+ 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 \(8 \times 10^6\) 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 \(\times 10^6\) 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 CD3e^-/^-\beta2m^-/^-H-2Db^-/^-H-2Kb^-/^- 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,
we transferred OT-1 cells into β2m−/−I-Ab−/− 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 β2m−/−I-Ab−/− mice, and CD8+ T cell numbers in two other mice were significantly less than in CD3ε−/−β2m−/−H-2Db−/−H-2Kb−/− 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 Kb-expressing hosts, peripheral survival/LDP of OT-1 cells is hindered by H-2Kb deficiency to 20-fold lower levels, further reduced to over 50-fold lower levels in CD3ε−/−β2m−/−H-2Db−/−H-2Kb−/− mice (Fig. 3 A), and even more reduced to 100-fold lower values and some times totally abrogated in β2m−/−I-Ab−/− 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 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 CD44high 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 CD3ε−/− 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 CD44highCD8+ 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 106), however, never reached the plateau obtained in CD3ε−/− 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.
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 ticking 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 as 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 TCRs 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+ and Ly5- mice were from Charles River/Iffa-Credo. TCR Tg monoclonal mice in a B6.Rag2−/− genetic background were: monoclonal αH-bearing a Vε−/− and MHC class I molecules (CD3ε−/−). 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 were prepared, and the number and phenotype of donor CD3+CD8+ (and in one case CD3+CD4+) +/− T cells was assessed. Since CD3−CD8− and 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, 3′ d after transfer.

BM chimeras. BM chimeras were made by lethal irradiation of host mice followed by BM transfection. We generated mice containing variable numbers of BM-derived cells expressing MHC class I, Tg KO mice were lethally irradiated (900 rads) with a 125I source and rescued by the i.v. injection of 5 × 10^7 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 CD3ε−/−/− Tg KO mice. Fig. S3 shows results obtained in MHC class I−/− Tg KO mice and MHC class I−/− Tg KO BM 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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