Self–class I MHC molecules support survival of naive CD8 T cells, but depress their functional sensitivity through regulation of CD8 expression levels

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Previous studies have suggested that naive CD8 T cells require self–peptide–major histocompatibility complex (MHC) complexes for maintenance. However, interpretation of such studies is complicated because of the involvement of lymphopenic animals, as lymphopenia drastically alters naive T cell homeostasis and function. In this study, we explored naive CD8 T cell survival and function in nonlymphopenic conditions by using bone marrow chimeric donors and hosts in which class I MHC expression is absent or limited to radiosensitive versus radioresistant cells. We found that long-term survival of naive CD8 T cells (but not CD4 T cells) was impaired in the absence of class I MHC. However, distinct from this effect, class I MHC deprivation also enhanced naive CD8 T cell responsiveness to low-affinity (but not high-affinity) peptide–MHC ligands. We found that this improved sensitivity was a consequence of up-regulated CD8 levels, which was mediated through a transcriptional mechanism. Hence, our data suggest that, in a nonlymphopenic setting, self–class I MHC molecules support CD8 T cell survival, but that these interactions also attenuate naive T cell sensitivity by dynamic tuning of CD8 levels.

The persistence of a functional pool of lymphocytes is critical to keeping the adaptive immune system ready to respond to pathogens and transformed cells. It is currently believed that maintenance of functional T cells in the periphery is an active process. However, the factors involved in regulating T cell homeostasis and functional reactivity are poorly understood, in part because these factors may vary depending on the T cell subset (e.g., CD4 or CD8 populations), their differentiation state (naive, effector, or memory), and the overall size of T cell pool (lymphoreplete versus lymphopenic; Marrack and Kappler, 2004).

For CD8 T cells, several studies compared the recovery of donor cells from class I MHC (MHC I)–deficient hosts to that from WT hosts, concluding that T cell interaction with MHC is critical for the survival of naive T cells, but irrelevant for memory T cells (Tanchot et al., 1997; Nesić and Vukmanović, 1998; Murali-Krishna et al., 1999; Sprent and Surh, 2002; Markiewicz et al., 2003; Surh and Sprent, 2005). However, a feature of these studies is the reliance on analysis of lymphopenic hosts to determine survival of naive CD8 T cells. As subsequent studies have indicated, lymphopenic conditions increase availability of homeostatic cytokines and can also drive proliferation and differentiation of naive T cells, through lymphopenia-driven homeostatic proliferation, which involves TCR engagement with self-peptide–MHC ligands (Ernst et al., 1999; Kieper and Jameson, 1999; Goldrath et al., 2002; Jameson, 2002, 2005; Marrack and Kappler, 2004). Consideration of the impact of lymphopenia in previous studies has reopened the debate about the role of MHC molecules in maintenance of both CD4 and CD8 T cells (Jameson, 2002, 2005; Dorfman and Germain, 2002; Germain et al., 2002; Grandjean et al., 2003; Martin et al., 2006). Indeed, studies suggest that, in the absence of class II MHC (MHC-II) molecules, naive CD4 © 2009 Takada and Jameson This article is distributed under the terms of an Attribution–Noncommercial–Share Alike license for the first six months after the publication date (see http://www.jem.org/misc/terms.shtml). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).
T cells survive efficiently in lymphoreplete hosts (Clarke and Rudensky, 2000; Dorfman et al., 2000), but decline in a lymphopenic environment (Takeda et al., 1996; Brocker, 1997; Rooke et al., 1997; Witherden et al., 2000; Labrecque et al., 2001; Polic et al., 2001; Dorfman and Germain, 2002; Germain et al., 2002; Jameson, 2002, 2005; Grandjean et al., 2003; Martin et al., 2006). Importantly, there have been no similar comprehensive studies of the requirement for MHC-I in maintenance of CD8 T cells under nonlymphopenic conditions. This is due, at least in part, to complications in adoptive transfer approaches; WT T cells transferred into MHC-I–deficient recipients are rejected because of a small host CD8 T cell pool highly responsive to MHC-I molecules (Ljunggren et al., 1995, 1996; Vugmeyster et al., 1998), a problem that is not encountered in transfer of WT cells in MHC-I–deficient hosts.

A related issue is the identity of the MHC–expressing host cell populations that are required for T cell survival. Data from Brocker (1997) suggested that MHC-II expression on DCs was essential for maintenance of CD4 T cells, and that restricted expression of MHC-I on DCs was sufficient for homeostasis of CD8 T cells (Gruber and Brocker, 2005). However, studies using BM chimeras suggested that class I expression on either radiosensitive or radioresistant cells was sufficient for maintenance of CD8 T cells (Markiewicz et al., 2003). Once again, however, interpretation of these data is complicated by the use of lymphopenic recipient animals.

Recent studies suggest self-peptide–MHC molecules may have more important effect on T cell function than simple survival. Stefaňová et al. (2002) showed that the exposure of naive CD4 T cells in MHC-II–deprived condition results in an immediate reduction in sensitivity to foreign antigens associated with the loss of basal phosphorylation and polarization of TCR. Likewise, in the absence of MHC-II ligands, memory CD4 T cells were maintained, yet became functionally impaired (as measured by skin graft rejection; Kassiotsis et al., 2002; De Riva et al., 2007). More recently, Fischer et al. (2007) showed a progressive loss of motility by naive CD4 T cells transferred into MHC-II–deficient hosts, leading to failed engagement with cognate antigen–bearing DCs in vivo. However, polar opposite results have also been reported. Naive CD4 T cells deprived of the contact with MHC–II were shown to exhibit a more vigorous Ca2+ influx after TCR triggering with high-affinity ligands (Smith et al., 2001), in parallel with decreased levels of CD5, which acts as a negative regulator of TCR signal (Tarakhovsky et al., 1995; Azzam et al., 1998, 2001; Smith et al., 2001). In addition, after a long-term maintenance in MHC-II–deficient condition, CD4 T cells acquired reactivity to syngeneic skin grafts (Bhandoola et al., 2002). Here again, the fact that experiments were performed under lymphopenic (Bhandoola et al., 2002) versus lymphoreplete (Fischer et al., 2007) conditions may contribute to these apparently contradictory results, but this has not been directly addressed.

CD8 is a coreceptor required to enhance the sensitivity of CD8 T cells, at least in part because of the activity of Lck bound to its intracellular domain (Zamoyska et al., 2003) and CD8’s ability to stabilize interactions between MHC-I and TCR (Garcia et al., 1996; Schott and Ploegh, 2002). CD8 expression levels are known to affect TCR signaling thresholds (Holler and Kranz, 2003; Maile et al., 2005; Feinerman et al., 2008), and it has been suggested that CD8 expression is not set in the thymus, but dynamically modulated in the periphery, contributing to the tolerance of naive cells and the prevention of redundant killing by effector cells (Rocha and von Boehmer, 1991; Maile et al., 2005; Xiao et al., 2007).

Recently, Park et al. (2007) reported that signals of common γ-chain cytokines, including IL–2, −7, and −15, increase CD8α expression at the transcriptional level. This action is impaired by signals via TCR, strikingly reflecting the TCR signal intensity (Park et al., 2007). However, the role of self-peptide–MHC-I for the CD8 tuning has not been directly examined.

In this study, we characterized the survival and function of naive CD8 T cells in MHC-I–deficient hosts under lymphoreplete condition. To allow for transfer of cells across an MHC-I mismatch barrier, we used BM chimeras for both donors and hosts in these experiments. We report that the long-term survival of naive CD8 T cells was impaired in MHC-I–deficient condition. However, naive CD8 T cells deprived of contact with MHC-I molecules showed phenotypic and functional evidence of retuning. This included elevated transcription and cell surface expression of CD8, which we show leads to enhanced responsiveness to low-affinity TCR ligands. These data demonstrate that engagement with self-MHC class I molecules, in a lymphoreplete environment, leads to divergent effects on CD8 T cell survival versus functional sensitivity.

RESULTS

Loss of MHC-I availability in a lymphoreplete setting leads to reduced long-term maintenance of naive CD8 T cells

Normal lymphocytes are rejected when transferred to unmanipulated MHC-I–deficient mice (including KdDb−/−, β2m−/−, and KdDb−/−β2m−/− animals) because of residual host CD8 T cells that respond vigorously to WT levels of MHC-I (Ljunggren et al., 1995, 1996; Vugmeyster et al., 1998; Boyman et al., 2006). Hence, we initially studied BM chimeric hosts that are partially deficient of “classical” class I MHC molecules (referred to as MHC-I) and are expected to be tolerant to WT donor cells. This included KdDb−/−→WT and reciprocal WT→KdDb−/− chimeras (WT→WT chimeras were also used as a control chimeric host). CFSE-labeled B6 donor cells were transferred and, 30 d later, analyzed for maintenance and division. Naive (CD44lo) donor CD8 T cells remained CFSEhi (Fig. 1a) and were maintained (Fig. 1b) equivalently in all hosts. As expected, the absolute number of donor naive CD4 cells recovered was comparable in all hosts, indicating that these chimeras were tolerant to MHC-I–positive donor cells (Fig. 1b). Importantly, the lack of proliferation of the donor naive T cells confirms that the hosts are functionally lymphoreplete. These data also suggest...
that maintenance of naive CD8 T cells in animals was similar whether the predominant expression of MHC-I molecules was on radiosensitive or radioresistant cells. These findings match the conclusions of Markiewicz et al. (2003) tracking was on radiosensitive or radioresistant cells. These findings whether the predominant expression of MHC-I molecules themselves, we used T cells from KbDb-/- mice (Tanchot et al., 1997; Nesic ´ and Vukmanovic ´, 1998; Markiewicz et al., 2003; Hao et al., 2006). We used KbDb-/- mice and various BM-chimeric recipients. 30 d after transfer into MHC-I–deficient hosts compared with KbDb-/- → WT hosts (in which MHC-I is expressed by radioresistant cells; Fig. 1 d). This impaired persistence was not observed for the cotransferred CD4 T cells, arguing against rejection or some generalized decrease in donor cell survival in KbDb-/- hosts. Nevertheless, the persistence of CD8 T cells at this frequency for 30 d in the absence of MHC-I is greater than might be predicted from previous studies (conducted in lymphopenic conditions) that suggested almost complete loss of naive CD8 T cells within 14 d after transfer into MHC-I–deficient mice (Tanchot et al., 1997; Nesic ´ and Vukmanovic ´, 1998; Markiewicz et al., 2003; Hao et al., 2006). We used KbDb-/- → WT chimeras as a MHC-I–expressing host to avoid potential NK rejection concerns that would be associated with transfer into WT hosts. Surprisingly, we observed rejection of KbDb-/- donor cells in WT → KbDb-/- hosts (unpublished data); thus, these chimeras were excluded in further studies. The half-life of naive CD8 T cells in MHC-I–deficient environments has been estimated to be between 2 and 7 d (Tanchot et al., 1997; Dorfman and Germain, 2002; Markiewicz et al., 2003), whereas studies in which TCR expression was postthymically ablated suggested a naive CD8 T cell half-life of 16–19 d (Labrecque et al., 2001; Polic et al., 2001). As previously discussed, interpretation of these studies is complicated by lymphopenia in the host animals. To track how host MHC-I influences naive CD8 T cell survival in lymphoreplete hosts, we determined the decay rate of donor KbDb-/- T cells in KbDb-/- and KbDb-/- → WT hosts until 60 d after transfer. Donor CD8 (and CD4) T cells were detected above background at all time points (Fig. 2 a); however, there was a clear difference in the maintenance of the donor CD8 pool in the MHC-I–deficient and –sufficient hosts. Although we estimated a half-life of 21 d for polyclonal CD8 T cells in KbDb-/- → WT hosts, the half-life was reduced to ~10 d in KbDb-/- hosts (Fig. 2 b), which is consistent with the data presented in Fig. 1 d. In contrast, the half-life for naive CD4 T cells was similar in the two hosts (Fig. 2 b). Our estimated half-lives for CD4 T cells suggested a faster decay rate than those reported in some other studies (Hataye et al., 2006; Polic et al., 2001). The reason for this difference is not clear, but does not appear related to use of chimeric hosts because we observed similar half lives using both chimeric and nonchimeric hosts (unpublished data), and our data are consistent.
with an overall average life span of peripheral naive T cells of 5–8 wk, as has been reported previously (von Boehmer and Hafen, 1993; Tough and Sprent, 1994). To control for variability in donor cell maintenance, we also assessed the ratio of donor CD8 to CD4 T cells in individual animals. As shown (Fig. 2c), the increased decay rate of CD8 cells in K\(^{d}D^{b}/-/- \) hosts also lead to a statistically significant difference in the donor CD8/CD4 ratio compared with the K\(^{d}D^{b}/-/- \rightarrow WT \) hosts.

Previous studies have shown that TCR affinity to self-peptide–MHC-I ligands (Kieper et al., 2004) or cross-reactivity with environmental ligands (Hao et al., 2006) can affect the homeostatic proliferation of T cells in lymphopenic condition, suggesting the different dependence on MHC requirement for homeostasis between clones. It was possible that the rapid decay rate of CD8 T cells in K\(^{d}D^{b}/-/- \) hosts led to selective survival of an oligoclonal pool of CD8 T cells, rather than

**Figure 2.** Impaired survival of CD8 T cells in the complete absence of MHC-I. Purified K\(^{d}D^{b}/-/- \) lymphocytes (Thy1.1) from K\(^{d}D^{b}/-/- \rightarrow WT \) BM chimeras were transferred (3–5 \( \times \) 10\(^6\)/mouse) to normal K\(^{d}D^{b}/-/- \) mice and K\(^{d}D^{b}/-/- \rightarrow WT \) BM chimeras. (a) Detection of donor CD8 T cells by flow cytometry at day 60 after transfer (left and middle). (right) Background events detected by the same method in nontransferred WT mice. Numbers above the outlined area indicate the percentage of cells in the lymphocyte gate. (b and c) Mice were sacrificed at indicated time points and analyzed for the recovery of donor T cells from the spleen and lymph nodes. (b) Absolute number of Thy1.1+CD44loCD8 (left) and Thy1.1+CD44loCD4 (right) donor cells. Half-lives (\( t_{1/2} \)) were calculated from regression lines (Hataye et al., 2006). (c) Combined results of two independent experiments. CD8/CD4 ratio was calculated before (R0) and after (R1) transfer into K\(^{d}D^{b}/-/- \ → WT \) BM chimeric or normal K\(^{d}D^{b}/-/- \) hosts. Difference of CD8/CD4 balance in donor cells between experiments was normalized by calculating R1/R0. (d) TCR V\( \beta \) analysis of Thy1.1+CD44loCD8 cells at day 20 after transfer. Percentage of cells that stain positive for each V\( \beta \) chain in total Thy1.1+CD44loCD8 cells is shown. The bars show the mean ± SD. All data other than c are representative of two independent experiments. *, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.001 \).
reflected decay of the bulk population. Arguing against this explanation, however, we found similar overall TCR repertoire diversity among surviving CD8 T cells at day 20 after transfer in both KβDβ−/− and KβDβ−/− → WT hosts, a time point where more rapid decay of CD8 T cells in the KβDβ−/− host is already apparent (Fig. 2 d).

Phenotypic analysis suggests retuning of CD8 T cells in MHC-I−deficient environments

We also examined how MHC-I deprivation influences expression of key cell surface molecules on donor CD8 (and CD4) T cells. Because IL-7 signaling plays a critical role for the survival of naive T cells (Akashi et al., 1997; Jameson, 2002, 2005; Marrack and Kappler, 2004; Surh and Sprent, 2005), we analyzed expression of IL-7Rα on T cells exposed to partial or complete MHC-I deficiency. Although CD8 and CD4 donor T cells exhibited comparable levels of IL-7Rα when maintained in WT or partially MHC-I−deficient hosts (Fig. 3 a and Fig. S1), there was a significant and selective down-regulation of IL-7Rα on CD8 T cells recovered from KβDβ−/− and KβDβ−/− → KβDβ−/− hosts (Fig. 3 b and Fig. S2). Decreased IL-7Rα staining was observed as a slight, but uniform, change in expression level on the MHC-I−deprived CD8 T cell pool (Fig. S2), arguing against

Figure 3. MHC-I−deprivation leads to phenotypic changes in naive CD8 T cells, suggestive of retuning. Lymphocytes from WT mice (a) or purified KβDβ−/− lymphocytes from KβDβ−/− → WT BM chimeras (b and c) were injected i.v. (3–5 × 10⁶/mouse) into indicated recipients. (a and b) 30 d after transfer, donor naive CD8 and CD4 T cells in host spleens were detected by CFSE label and a CD44lo naive T cell marker. Surface expression of IL-7Rα, CD5, and coreceptors (CD8α or CD4) was analyzed. (c) At day 20 after transfer, spleens and lymph nodes were harvested from host mice, and CD8α expression on CD44loCD8 donor cells (Thy1.1+) that stain positive for each Vβ chain was analyzed. Data are presented as relative fluorescence intensity (RFI), indicating the expression which is normalized to the average of mean fluorescence intensity (MFI) in WT → WT hosts (a) or KβDβ−/− → WT hosts (b and c) defined as 100. Data are mean ± SD. Results are representative of two independent experiments with at least three mice per group. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
progressive loss of IL-7Rαhigh cells. We also studied expression of CD5, a monomeric glycoprotein that negatively regulates the signaling through TCR and whose level on naive CD4 T cells has been observed in association with recent exposure to self-MHC molecules (Polic et al., 2001; Smith et al., 2001; Fischer et al., 2007). Similar to IL-7Rα, CD5 levels were substantially reduced on CD8 T cells recovered from KβDb−/− hosts (Fig. 3a and b, Fig. S1, and Fig. S2). These data are consistent with studies on MHC-II−deprived CD4 T cells, which were also shown to down-regulate CD5 (Polic et al., 2001; Smith et al., 2001; Fischer et al., 2007).

In contrast to these trends, CD8 itself was up-regulated on CD8 T cells isolated from hosts that were partially or completely deficient of MHC-I (Fig. 3a and b). Although an increase in CD8 expression was observed even when MHC-I was available on either radiosensitive or radioresistant cells, the effect was more pronounced in the hosts where radioresistant cells are deficient of MHC-I (Fig. 3a and Fig. S1), showing a similar extent of elevated CD8 expression as cells recovered from hosts that completely lack MHC-I (Fig. 3a and b, Fig. S1, and Fig. S2). CD4 expression on cotransferred CD4 T cells was not markedly altered in any host (Fig. 3a and b, Fig. S1, and Fig. S2). The CD8 levels were increased to a maximum of ~125% on CD8 T cells in MHC-I−deficient hosts. This elevation in CD8 expression in KβDb−/− hosts was observed for donor cells bearing different Vβ elements (Fig. 3c), implying that T cells of diverse specificity up-regulated the coreceptor under these conditions. On the other hand, TCR expression levels did not increase on donor T cells in MHC-I−deficient hosts (Fig. 3c). These changes in CD5, CD127, and CD8 expression were also seen in nonchimeric KβDb−/− hosts at day 20 (Fig. 2b, Fig. 3c, and not depicted).

Figure 4. MHC-I deprivation causes enhanced CD8 T cell reactivity to low-affinity TCR ligands in vitro. Purified CD8 T cells from KβDb−/−Rag−/−OT-I Tg → WT chimeras were CFSE stained and transferred (3 × 106/mouse) to KβDb−/− → KβDb−/− or KβDb−/− → WT BM chimeric hosts. All experiments were performed 14 d after transfer. (a) Spleens were harvested from recipients and analyzed for the expression of CD5 and CD8 on the surface of donor OT-I cells by flow cytometry. Data are presented as relative fluorescence intensity (RFI) normalized to the average of MFI in KβDb−/− → WT hosts. (b–d) Host spleen cells were cultured with irradiated WT splenocytes pulsed with different concentrations of OVAp (0–10^6 pM; b and d) or indicated peptides (c). Cells were analyzed for the expression of CD69 (b and c) or intracellular TNF (d). (e) Recipients were injected i.v. with WT DCs (10^6/mouse) pulsed or nonpulsed with OVAp. 18 h after DC injection, donor cells in the spleens (SPL) and lymph nodes (LN) were analyzed for the expression of CD69. The percentage of donor cells with CD69high phenotype is shown (b–d). Data represent mean ± SD. Data are representative of two to four independent experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
CD8 T cells are hyperreactive to low-affinity ligands after long-term exposure to MHC-I–deficient condition

The changes in CD5 and CD8 expression after MHC-I deprivation might alter CD8 T cell reactivity. To test this, we generated K6D-/- OT-I CD8 T cells (using K6D-/- Rag-/- OT-I → WT BM chimeras) as a source of cells with known TCR specificity. Mature OT-I CD8 T cells from these animals were then transferred into K6D-/- → K6D-/- and K6D-/- → WT BM chimeras, which were completely and partially MHC-I deficient, respectively. Based on the half-life determinations for polyclonal CD8 T cells (Fig. 2), we anticipated that OT-I cells would be maintained in both hosts for at least 14 d, and indeed we observed no differences in OT-I T cell numbers in the two hosts by this time point (not depicted). Also, similar to our data with polyclonal CD8 T cells, OT-I cells maintained in completely K6D-/- hosts showed increased CD8 expression and decreased CD5 levels, whereas TCR expression levels were unchanged compared with K6D-/- → WT controls (Fig. 4 a). We have previously defined the response of OT-I T cells to the strong agonist peptide OVA peptide (OVAp), as well as to weak agonist variants of OVAp (Jameson et al., 1993; Hogquist et al., 1994; Rosette et al., 2001). To test functional reactivity of these cells in vitro, OT-I cells from both hosts were stimulated with B6 splenocytes coated with various peptides, and up-regulation of the activation marker CD69 was monitored.

Studies using CD4 T cells have led to contradictory conclusions, with some studies suggesting MHC-II deprivation leads to enhanced functional reactivity (Smith et al., 2001; Bhandoola et al., 2002), whereas other works suggest it causes reduced sensitivity for cognate antigens (Kasisiotis et al., 2002; Stefanová et al., 2002; De Riva et al., 2007). Distinct from either of these findings, we found that the complete MHC-I deprivation had no effect on the functional sensitivity of OT-I cells toward OVAp, which is a high-affinity peptide for the OT-I TCR (Jameson et al., 1993; Hogquist et al., 1994), as determined by a dose-response assay (Fig. 4 b).

On the other hand, it has been proposed that engagement with self-peptide–MHC may tune the activation thresholds of T cells, to avoid autoreactivity and/or to discriminate the quantitative or qualitative differences of antigens (Grossman and Paul, 2001). Therefore, we asked whether host MHC-I environment can affect the donor T cell sensitivity to various strengths of TCR stimulation in vitro. We tested two variants of the OVAp, termed N6 and G4, which have strong and weak agonist activity, respectively, for OT-I (Jameson et al., 1993; Hogquist et al., 1994; Rosette et al., 2001). In addition, we tested the peptide β-Cat, which encodes a naturally occurring TCR antagonist for the OT-I TCR (Santori et al., 2002), to determine if reactivity to this self-peptide was altered after MHC-I deprivation.

Although no change in response to the strong agonist peptide N6, or the antagonist peptide β-Cat were noted (Fig. 4 c), we found that MHC-I–deprived OT-I cells consistently showed an improved functional response toward the weak agonist G4 (Fig. 4 c). This finding suggests that MHC-I deprivation leads to enhanced reactivity toward weak agonists, although it was not sufficient to induce overt reactivity toward self-peptide–MHC ligands (as determined by the minimal response toward β-Cat and endogenous peptide–MHC complexes displayed by “no-peptide” control B6 APCs).

Although others have used CD69 up-regulation to determine altered T cell sensitivity after MHC-deprivation (Bhandoola et al., 2002), one study reported that MHC-II deprivation of CD4 T cells did not impact sensitivity as measured by CD69 induction, but did impair cytokine production (Stefanová et al., 2002). We also assayed induction of TNF after in vitro stimulation. Similar to CD69 induction, production of TNF in response to the strong agonist OVAp was not affected by MHC-I deprivation (Fig. 4 d). Stimulation using the G4 peptide induced only weak and variable TNF production (unpublished data), and thus this assay could not be used reliably to measure functional sensitivity to this low-affinity ligand.

Together, these data suggest that preventing the engagement of CD8 T cells with self-peptide–MHC-I (in a non-lymphopenic environment) did not impact overall TCR sensitivity of the OT-I CD8 T cells, but did selectively enhance their responses to low-affinity TCR ligands. However, our data suggest that these changes in sensitivity did not extend to overt reactivity toward self-peptide–MHC ligands.

Fischer et al. (2007) found that MHC-II deprivation of CD4 T cells had little impact on their functional sensitivity in vitro, but led to a dramatic impairment of responsiveness in vivo caused by compromised motility, which prevented engagement of DCs bearing strong agonist ligands. To explore whether analogous changes were occurring in the CD8 T cell system, we maintained K6D-/- OT-I T cells in K6D-/- → K6D-/- or K6D-/- → WT BM chimeras for 14 d, and then injected these animals with WT DCs that were pulsed with OVAp (or PBS). The response of the OT-I pool was assessed by CD69 up-regulation 18 h later (Fig. 4e). A robust response was induced in both host environments, and there was no indication that the MHC-I deprived OT-I cells showed a reduced reactivity toward antigen-bearing DC—indeed, there was a trend (although not statistically significant) toward the opposite conclusion. In contrast to the findings for MHC-II–deprived OT-II T cells (Fischer et al., 2007), removal of MHC-I ligands did not impair OT-I sensitivity toward high affinity antigen-bearing DCs in vivo.

We concluded that both in vitro and in vivo responsiveness of CD8 T cells to stimulation with potent peptide–MHC ligands did not change dramatically, even after long-term exposure of CD8 T cells to MHC-I–deficient condition. This was despite the phenotypic alterations (CD5 decrease and CD8 increase) indicative of T cell retuning. Importantly, our in vivo studies did not observe the marked impairment in reactivity described for MHC-II–deprived CD4 T cells (Fischer et al., 2007). At the same time, we found that the ability of CD8 T cells to qualitatively discriminate antigenic stimulation was altered in MHC-I–deficient
Figure 5. CD8 T cell retuning occurs rapidly after MHC-I deprivation. (a–c, e, and f) Purified CD8 T cells from WT OT-I Tg mice (Thy1.1) were transferred (3 × 10^6/mouse) to WT or K^Dβ^−/− mice. 1 d (a–c) or 2 h (e and f) after transfer, host spleen cells or pooled lymph node cells were analyzed for the phenotype of donor OT-I cells (a and e) or were stimulated with irradiated WT splenocytes pulsed with different concentrations of OVAp (0–10^6 pM; b) or indicated peptides (c and f). (d) Purified CD8 T cells from K^Dβ^−/− Rag^−/− OT-I Tg → WT chimeras were CFSE stained and transferred (3 × 10^6/mouse) to K^Dβ^−/− → K^Dβ^−/− or K^Dβ^−/− → WT BM chimeric hosts. 1 d later, host mice were injected (i.v.) with WT DCs (10^6/mouse) that were pulsed or nonpulsed with OVAp. 18 h after DC injection, donor cells in the spleens (SPL) and lymph nodes (LN) were analyzed by flow cytometry. The percentage of donor cells with CD69hi phenotype after stimulation is shown (b–d and f). (g and h) Purified OT-I cells with different congenic markers (Thy1.1+Thy1.2+ and Thy1.1+Thy1.2−) were separately transferred (4–5 × 10^6/mouse) to WT and K^Dβ^−/− mice. Cells were CFSE-labeled before transfer in h. 36 h after transfer, lymph nodes cells from both hosts and irradiated B6 spleen cells were mixed at equal number and cultured with or without G4. Cells were analyzed for...
conditions, with the cells showing enhanced reactivity toward low-affinity ligands.

**Short-term MHC-I deprivation leads to changes in phenotype and tissue-restricted changes in CD8 T cell sensitivity**

We had previously suggested that the discordant conclusions from studies on the effect of MHC-II deprivation on naive CD4 T cell responses might arise from differences in the time points studied (Hogquist et al., 2003). Thus, the study reporting that MHC-II deprivation diminished T cell sensitivity assayed the cells within the first 24 h (Stefanová et al., 2002), whereas other studies (which reported increased T cells sensitivity after MHC deprivation) looked at later time points (Smith et al., 2001; Bhandoola et al., 2002). These data could be consistent with an adaptation of T cells to the loss of self-peptide–MHC ligand engagement, which leads to a reversal in T cell sensitivity when comparing short- and long-term MHC deprivation (Hogquist et al., 2003).

To test this model, we modified our assays to examine OT-I sensitivity after short-term MHC-I deprivation for only 1 d. We examined the same adoptive transfer system (with K\(^{b-/-}\) OT-I cells placed into K\(^{b-/-}\) or K\(^{b-/-}\) → WT BM chimeras). In addition, we took advantage of the fact that short-term transfer of WT cells into K\(^{b-/-}\) hosts does not result in their elimination (Fig. S3) and also assayed OT-I cells adoptively transferred into intact WT K\(^{b-/-}\) hosts. In both settings, MHC-I deprivation led to loss of CD5 and IL-7Rα expression in the spleen and lymph nodes by 24 h after transfer (Fig. 5 a and not depicted). In the same hosts, CD8 levels were increased (by \(\sim 25\%\)), but this effect was more pronounced in the lymph nodes than in the spleen (Fig. 5 a and not depicted). These data suggested that the phenotypic adaptation of CD8 T cells to MHC-I deprivation occurs rapidly and, unexpectedly, that changes in CD8 expression were most pronounced in the lymph node.

To assess the impact which MHC-I deprivation for 24 h had on functional sensitivity, we again tested responses to OVAp and its variants. As with cells deprived of MHC-I for 14 d, there was no change in responses to the high-affinity OVAp ligand, which were measured in vitro (Fig. 5, b and c) or in vivo (Fig. 5 d). However, reactivity to G4 peptide-coated APC was enhanced (Fig. 5 c). Interestingly, this effect was detected in OT-I cells derived from the lymph nodes, but not from the spleen (Fig. 5 c).

These data suggest that sensory adaptation of CD8 T cells deprived of MHC-I takes place rapidly, within 1 d. It was possible that even this time frame was too long to observe rapid changes in CD8 T cell sensitivity. Studies with CD4 T cells suggested that loss of contact with MHC-II molecules for as little as a few hours was sufficient to compromise functional reactivity (Stefanová et al., 2002). We also studied OT-I cells 2 h after adoptive transfer into B6 versus K\(^{b-/-}\) hosts. At this time point, no consistent changes in expression of CD5 or CD8 were observed, and no changes in OVAp or variant peptide reactivity were detected (Fig. 5, e and f).

To extend these studies, we used additional functional assays. First, we tested whether the enhanced responses of MHC-I–deprived OT-I cells would be observed in the presence of normal OT-I cells. Congenically marked OT-I cells were transferred into class I–deficient or normal hosts, and 36 h later the two populations were pooled and stimulated with G4 peptide in vitro. Because both normal and MHC-I–deprived OT-I cells are stimulated in the same culture, this assay controls for potential differences in APC populations and allows for direct comparison of the OT-I cell responses. Consistent with our previous studies, G4/K\(^{b}\) induced CD69 expression was significantly enhanced on OT-I cells recovered from MHC-I–deficient hosts (Fig. 5 g). We also investigated induction of signal transduction intermediates. In an earlier study, we characterized induction of phosphorylated c-Jun (pc-Jun) by G4/K\(^{b}\) stimulation OT-I cells (Rosette et al., 2001). Analysis of this response also revealed an enhanced response by MHC-I deprived OT-I cells (Fig. 5 g). Both of these assays monitored early activation responses, and we also tested proliferation, as a late functional response. Using a CFSE dye dilution assay, we observed similar proliferative potential of OT-I cells derived from either normal or MHC-I–deficient environments (Fig. 5 h). This result might suggest that the effects of MHC-I deprivation do not affect all responses equally, and/or that these effects are transient and are lost during longer term functional assays. This issue is explored further in the Discussion.

Hence, our studies suggested that the phenotypic and functional changes associated with MHC-I deprivation of CD8 T cells arose between 2 and 24 h (and was maintained for at least 14 d), and led to enhancement of early functional responses to low-affinity ligands.

**Enhanced reactivity of MHC-I–deprived T cells is attributable to increased CD8 levels**

Our studies showed that OT-I T cell transfer into MHC-I–deficient hosts caused both enhanced reactivity to the weak agonist G4/K\(^{b}\) and an increase in CD8 expression levels (Figs. 4 and 5). Previous studies have suggested that changes in CD8 expression or accessibility cannot ably affect T cell sensitivity and ligand binding, especially for low-affinity peptide–MHC ligands (Jameson et al., 1994; Holman et al., 2005; Daniels et al., 2006; Park et al., 2007; Xiao et al., 2007; Feinerman et al., 2008), but the observation that MHC-I
deprivation only lead to an increase of ~25% in CD8 levels (Figs. 4 a and 5 a) left open the question of its mechanistic relevance for the changes in T cell sensitivity. Thus, we characterized how small changes in CD8 accessibility affected OT-I reactivity by stimulating freshly isolated OT-I cells with G4 or OVAp in the presence of monoclonal anti-CD8β (clone 53–5.8) antibodies titrated over a wide concentration range (Fig. 6 a). The same antibody was used as a fluorescent conjugate to determine the levels of “unblocked” CD8, presumably available for ligand binding.

Although the OT-I response to high-dose OVAp peptide was barely affected by anti-CD8β blockade, the response to G4 peptide was drastically impaired, even when CD8 levels were only partially reduced (Fig. 6 b). As has been suggested by other studies (Holler and Kranz, 2003; Daniels et al., 2006), these data suggest that low-affinity ligand engagement and/or reactivity is strongly influenced by the availability of the CD8 coreceptor. However, the OT-I response to OVAp does involve CD8 because stimulation of OT-I cells with low doses of OVAp is highly sensitive to CD8 blockade (Fig. 6 c), indicating that this blockade becomes inefficient when TCR stimulation is in excess.

Anti-CD8α antibody (clone 53–6.7) showed blocking properties similar to 53–5.8 (Fig. S4, a and b), although they were slightly less effective (Fig. S4 c). These results might at first appear to contradict previous studies (including our own; Daniels and Jameson, 2000) showing that 53–6.7 enhances binding of cognate peptide–MHC tetramers to OT-I cells, whereas 53–5.8 blocks this binding. This can be explained by the capacity of the CD8 antibodies, under physiological conditions, to induce capping and internalization of CD8; however, this will not occur in the flow cytometric assays used to measure peptide–MHC tetramer binding.

We next used the same approach to determine whether the enhanced CD8 expression levels seen on class I–deprived OT-I cells could account for their improved G4/Kb sensitivity. At 36 h after adoptive transfer, donor OT-I cells exhibited an ~15% increase in CD8 expression when recovered from the lymph nodes of KbDb−/− hosts compared with WT hosts (Fig. 6 c, no Ab), and the class I–deprived population showed enhanced reactivity toward G4 (Fig. 6 d, left). Treatment of the class I–deprived OT-I cells with low dose (1/102,400) of the anti-CD8β antibody resulted in available CD8 levels being reduced to those found in the WT group (Fig. 6 c). Interestingly, this antibody treatment lead to a corresponding decrease in reactivity toward G4, resembling the response of OT-I cells from a WT host (Fig. 6 d, left). As expected, the response to high-dose OVAp was not affected by either the MHC-I expression status of the host, or partial CD8 blockade (Fig. 6 d right). Once again, the anti-CD8α antibody, 53–6.7, yielded similar results (Fig. S4, d and e). Hence, these results suggested that MHC-I–deprivation led to enhanced OT-I T cell reactivity primarily because of increased CD8 expression levels.

**MHC-I deprivation regulates CD8 levels at the level of transcription**

We next sought to determine the mechanism by which CD8 levels are regulated by exposure to MHC-I molecules. At least two models can be imagined. In the first, MHC-I deprivation induces up-regulation (transcriptional or translational) of CD8. In the second model, encounter of CD8 with self-MHC-I molecules might lead to internalization of cell surface CD8.

To test these models, we developed an in vitro system where CD8 T cells could be more easily manipulated. T cells were cultured overnight in the presence or absence of anti-Kb and anti-Dα monoclonal antibodies. Interestingly, such treatment revealed that anti–MHC-I blockade led to up-regulation of CD8α and CD8β levels on CD8ve T cells from both polyclonal B6 (Fig. 7 a) and OT-I (Fig. 7 b) animals.
This was not a nonspecific effect because expression of CD4 by polyclonal B6 T cells was unaffected (Fig. 7 a). Furthermore, cell treatment with anti-CD45.2 antibodies (which, like anti-class I antibodies, would bind all cells in the culture) did not affect CD8 expression levels (unpublished data). Thus, anti-class I antibody blockade appears to specifically up-regulate CD8 expression levels. Notably, the elevation in CD8 levels was completely abrogated when actinomycin D or cycloheximide (which inhibit transcription and protein synthesis, respectively) were added to the in vitro cultures. These data suggest that the increase in CD8 expression after MHC-I blockade is caused by altered transcription.

As an alternative assay for transcriptional regulation, we made use of a CD8α gene reporter transgenic strain termed E8I-CD4 (Park et al., 2007; Adoro et al., 2008). This well defined reporter expresses mouse CD4 under regulation of both the CD8α promoter and E8I enhancer elements, and is active in mature CD8CD4− thymocytes and peripheral CD8 T cells (Park et al., 2007; Adoro et al., 2008). Hence, changes in transgenic CD4 expression would indicate altered CD8α gene expression, but, because CD4 does not engage MHC-I molecules, CD4 internalization would not be affected by MHC-I blockade. Thus, we cultured the spleen cells from E8I-CD4 Tg mice in vitro with anti-Kb and anti-Db antibodies. As expected, the cell surface expression of endogenous CD8α and CD8β was enhanced by this treatment, but we also observed a significant increase in expression of the E8I-CD4 transgene (whereas endogenous CD4, on other cells in the same culture, was not affected; Fig. 7 c). As expected, addition of actinomycin D or cycloheximide prevented these changes in E8I-CD4 (as well as CD8α and CD8β) levels (unpublished data). Together, these data suggest that up-regulation of CD8 after deprivation of MHC-I contact is chiefly a result of increased CD8α transcription, operating (at least in part) through the E8I enhancer and/or CD8α promoter.

The capacity to induce up-regulation of CD8 expression by in vitro anti-MHC-I blockade also offered the opportunity to test whether this effect was reversible after reexposure to self-MHC ligands in vivo. OT-I CD8 T cells were cultured with or without anti-Kb/Dd blockade, and then transferred into normal B6 hosts for 48 h. As expected, in vitro MHC-I blockade lead to CD8α and CD8β up-regulation (Fig. 7 d, Before), but this effect was lost after transfer into normal hosts (Fig. 7 d, 48 h). These data suggest that the increase in CD8 expression induced by impaired MHC-I interactions is not a permanent feature of the cells, and that CD8 levels can “retune” once the T cells have the opportunity to reengage self-ligands.

**DISCUSSION**

The role of self-peptide–MHC class I complexes in survival and function of naive CD8 T cells has been difficult to address. This is in large part caused by donor–host histocompatibility issues, which have typically been solved by the use of lymphopenic hosts for adoptive transfer experiments. With the understanding that lymphopenia-driven homeostatic proliferation affects T cell proliferation, differentiation, and function (Dorffman and Germain, 2002; Germain et al., 2002; Grandjean et al., 2003; Jameson, 2005; Martin et al., 2006),
these studies must be interpreted with care. Alternative approaches have used TCR ablation by genetic means, including cre-lox TCR gene deletion (Polic et al., 2001) and tet regulatable TCRα transgene expression (Labrecque et al., 2001). However, conclusions from these studies are also complicated because T cell development is terminated in such systems, and because these approaches test the role of the TCR itself, rather than its encounter with self-MHC molecules, during T cell maintenance. In the current study, we make use of established radiation BM chimeras and short-term adoptive transfer experiments to examine how the presence of MHC-I impacts CD8 T cell maintenance under lymphorenplete conditions.

Our data on naive CD8 T cell survival in the absence of classical MHC-I molecules suggest a much more gradual decline than has previously been reported. We find that the CD8 T cell half-life changed from ~21 to ~10 d in WT versus KdDβ−/− hosts, respectively. Earlier studies reported fast decay rates in MHC-I–deficient hosts, with almost complete disappearance of donor CD8 T cells by 2–14 d (Tanchot et al., 1997; Markiewicz et al., 1998, 2003; Hao et al., 2006). However, such studies were conducted in lymphopenic hosts that would allow at least some CD8 T cells to undergo efficient homeostatic proliferation in the WT host. In addition, most previous studies used MHC-I–expressing donor CD8 T cells in their studies, which would allow for MHC-I recognition after T–T interactions. The absence of CFSE dye dilution in any of the hosts in our study rules out homeostatic proliferation as a basis for their improved maintenance in MHC-I–deficient hosts, but the basis for naive CD8 T cell decline in MHC-I–deficient hosts is not yet clear. Interestingly, we observed a reduced expression of the IL–7Rα chain (CD127) on MHC-I–deprived CD8 T+ cells. Because IL–7 is a critical survival factor for naive T cells (Akashi et al., 1997; Jameson, 2002, 2005; Marrack and Kappler, 2004; Surh and Sprent, 2005), reduced CD127 levels may suggest decreased survival potential. This interpretation is complicated, however, by the finding that CD127 expression is reduced after its engagement by IL–7 (Park et al., 2004), so low CD127 levels can also be a result of efficient IL–7 encounter. Despite the more rapid decay rate of CD8 T cells in MHC-I–deficient environments, a small number of CD8 T cells clearly survived as late as 60 d after transfer (Fig. 3, a and b). This could be caused by a uniform decay rate of the entire population or reflect variability in the requirement for MHC-I recognition, such that some clones are highly sensitive to MHC-I deprivation, whereas others are independent of self-MHC-I ligands. In our studies using OT-I T cells, we noted that their survival was equivalent by day 14 after transfer into MHC-I–deficient and control hosts (unpublished data), suggesting that not all clones follow the same decay kinetics. However, analysis of TCR Vβ usage could not demonstrate a significant bias in the overall repertoire of polyclonal CD8 T cells persisting to day 20 after transfer into WT versus KdDβ−/− hosts. Nevertheless, further studies will be required to determine whether all CD8 T cells exhibit similar requirements for self-MHC-I for their maintenance. Importantly, our data suggesting a role for MHC-I in the maintenance of polyclonal CD8 T cells contrasts with strong evidence for equivalent survival of CD4 T cells in MHC-II–deficient and –sufficient hosts (under lymphorenplete conditions; Clarke and Rudensky, 2000; Dorfman et al., 2000). TCR ablation studies also report faster decay rates for naive CD8 T cells versus CD4 T cells (Labrecque et al., 2001; Polic et al., 2001). Together with our data, these findings suggest distinct requirements for TCR-mediated survival signals within the naive CD4 and CD8 populations.

By use of distinct BM chimeric hosts, we unexpectedly found a differential role of MHC-I expressed on hematopoietic and nonhematopoietic cells. Although MHC-I expression in either compartment appeared sufficient to mediate normal CD8 T cell survival, changes in CD8 expression level were more marked when MHC-I was lacking on radioresistant cells (Fig. 3). Furthermore, at short time points, phenotypic and functional aspects of CD8 tuning were more apparent in lymph nodes than in spleen (Fig. 5, a and c). At steady state, T cells are thought to migrate within the T cell zones of secondary lymphoid organs along a network formed by nonhematopoietic stromal cells (Bajenoff et al., 2006), and it was recently reported that IL–7 is mainly produced by fibroblastic reticular stromal cells (gp38CD31+CD35−) in T cell zones of secondary lymphoid organs (Link et al., 2007). It is interesting to speculate that such radioresistant stromal cells may offer both IL–7 and self-peptide–MHC-I molecules to circulating CD8 T cells, and thus regulate the T cell’s tuning.

Previous studies on the impact of self-MHC molecules on T cell sensitivity have yielded strikingly divergent results. Because of the issues with CD8 T cell rejection discussed, such studies have mostly focused on CD4 T cells. Published works indicated that MHC-II–deprived CD4 T cells have elevated (Bhandoola et al., 2002; Smith et al., 2001) or diminished (Stefanová et al., 2002) sensitivity to antigen, or that intrinsic antigen sensitivity is intact in such cells, but their in vivo reactivity is impaired because of lost motility (Fischer et al., 2007). These studies varied in their use of lymphopenic hosts, but also covered a range of time points (from hours to months) in the analysis of CD4 T cell sensitivity. Therefore, it was possible that the discrepancies between the studies could be explained by suggesting that MHC deprivation caused initial desensitization of T cell sensitivity (involving lost basal TCR signaling and polarization of TCR), followed by a compensatory retuning (involving reduced CD5 expression) allowing for improved functional sensitivity (Hoggquist et al., 2003). Although such a model may still apply to CD4 T cells, our data with CD8 T cells suggest a distinct model, in that MHC-I deprivation (whether for 2 h, 24 h, or 14 d) did not impact sensitivity to strong agonist peptide–MHC ligands in vitro or in vivo (Fig. 4, b–e). However, sensitivity to weak agonist ligands, assessed in vitro, was enhanced after MHC-I deprivation for as little as 24 h (Fig. 5 c), and this increased reactivity was maintained for

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at least 2 wk (Fig. 4 c). Although we could confirm and extend previous works suggesting self-MHC deprivation results in decreased CD5 levels (Figs. 4 a and 5 a; Smith et al., 2001), our data also indicated that the functionally relevant change is enhanced CD8 expression. We observed a modest, but reproducible, increase in CD8 expression by OT-I cells in KdDb hosts, which correlated with their improved reactivity toward the weak agonist G4/Kb (Fig. 5, a and c), and could demonstrate that mild blockade of CD8 expression levels restored G4/Kb reactivity (Fig. 6 and Fig. S4), supporting the model that CD8 fine tuning can explain the differential reactivity to low-affinity peptide–MHC ligands.

The observation that MHC-I–deprived OT-I T cell responsiveness to the strong agonist OVA/Kb was not affected even at low doses is consistent with a selective role for CD8 tuning in qualitative regulation of low-affinity TCR ligands. CD8 plays an important role in TCR binding and reactivity for peptide–MHC ligands, but this is more profound for low-affinity ligands (Holler and Kranz, 2003; Holman et al., 2005; Maile et al., 2005). Furthermore, some data suggest the stability of low-affinity TCR interactions required for thymic positive selection is highly dependent on CD8 (Daniels et al., 2006). Hence, our data are consistent with the model that MHC-I deprivation causes a compensatory change in CD8 levels, as the cells “cast around” for the low-affinity self-peptide–MHC ligands that sustain their basal TCR signaling.

Initially, it might appear our data contradict studies showing that engagement with nonstimulatory peptide–MHC ligands (including self-peptide–MHC molecules) can enhance CD8 T cell responses to agonist ligands (Michelleti et al., 2000; Cebeckerug u et al., 2005; Yachi et al., 2005, 2007). However, our studies explore how MHC-I deprivation affects subsequent CD8 T cell responses, whereas those previous works test effects of simultaneous exposure to agonist and “coagonist” ligands. Indeed, because our functional studies use WT APCs (expressing the normal array of self-peptide–MHC ligands), it is quite feasible that the enhanced sensitivity we observe in the response to low-affinity agonists actually reflects enhanced recognition of self-coagonist ligands. Along these lines, it is worth noting that the cooperative effect of coagonists is most pronounced in the response to low affinity agonists and at early time points during activation (Yachi et al., 2007), similar to our observations for MHC-I–deprived CD8 T cells. It will be interesting to explore this model in future studies.

The mechanism by which MHC-I deprivation resulted in CD8 up-regulation was also explored. Our data revealed that blockade of MHC-I molecules lead to increased transcriptional activity at the CD8α gene, as revealed by use of transcriptional inhibitors and the E8–CD4 reporter strain (Fig. 7, b and c). CD8β is also elevated on MHC-I deprived CD8 T cells, and it is possible that this is also regulated transcriptionally. However, previous studies have shown that the requirement for mouse CD8b to associate with CD8α for surface expression leads to CD8β expression levels being strongly dependent on the expression level of Gld8α (Gorman et al., 1988; Park et al., 2007). Hence, our data suggest that loss of encounter with self-MHC-I molecules causes naive CD8 T cells to increase transcription and surface expression of CD8.

Our data also indicated that “tuning” of CD8 levels was reversible: CD8 T cells deprived of encounter with MHC class I up-regulated CD8, but upon reexposure to a normal environment CD8 expression levels were restored to normal (Fig. 7 d). This capacity for “retuning” CD8 expression levels might affect our ability to determine functional consequences of MHC-I deprivation. As shown in (Fig. 5, g and h), MHC-I deprivation led to enhanced early functional responses (induction of CD69, pc-Jun) but no changes in proliferation, in response to G4/Kb. However, because these assays must (to be physiologically relevant) involve exposure of responding T cells to MHC-I molecules, retuning may occur during the assay, complicating interpretation of later responses (including proliferation). Analysis of other early activation events is complicated by the inability of G4/Kb to induce detectable proximal activation in OT-I cells (Rosette et al., 2001). Although further studies will be needed to explore this concept further, these data suggest that CD8 T cells might constantly fine-tune their CD8 expression levels to match their recent encounter with self-peptide–MHC class I ligands.

The capacity of mature CD8 T cells to regulate their ligand binding and functional reactivity by dynamic “coreceptor tuning” has become clear over recent years (Maile et al., 2005; Park et al., 2007; Xiao et al., 2007). Singer’s group showed that responsiveness to IL-7 enhances CD8α transcription, and that this pathway is blocked by TCR encounter with strong agonists (Park et al., 2007). Furthermore, terminating self-MHC interactions during thymic development enhanced CD8 expression levels (Park et al., 2007). Our data extend this model of Park et al. by focusing on the fate of mature peripheral CD8 T cells acutely deprived of self-peptide–MHC-I molecules. In their work, the effect of IL-7 on coreceptor expression has been attributed to the activity of one of the enhancer elements, E8$, specifically used in mature CD8 T cells (Park et al., 2007). It is interesting to note the fact that enhanced transcriptional regulation of the E8I–CD4 reporter system was seen in response to both encounter with IL-7 (Park et al., 2007) and MHC-I deprivation (Fig. 7 c). Whether both systems involve the same elements within the CD8α promoter or E8$ enhancer is not clear, but it is tempting to speculate that the regulatory processes could be overlapping. Our data showed that terminating the interaction with MHC-I ligands led to elevated CD8 expression, but decreased IL-7Rα expression (Figs. 2 e, 5 a, and S2). As discussed earlier, impaired IL-7R expression is difficult to interpret, because it may arise by either reduced IL-7R expression or elevated IL-7R signaling—both of which would involve decreased CD127 transcription and expression. Our data might indicate that deprivation of the basal interaction with self-MHC-I directly leads to impaired CD127 expression, which is an interesting contrast to the impact of strong TCR signals, which also leads to loss of CD127 expression.
Further studies will be required to investigate whether prolonged loss of self-MHC encounters directly lead to reduced expression of IL-7Ra, and whether this in turn accounts for the impaired long-term survival of CD8 T cells in KdDb-/- hosts.

In conclusion, we show the first evidence that self-peptide–MHC-I complexes are required for efficient survival of polyclonal CD8 T cells in lymphoreplete hosts. At the same time, self-peptide–MHC-I encounter also impairs CD8 T cell responsiveness toward low-affinity peptide–MHC antigens through down-regulation of CD8, which may contribute to maintenance of T cell tolerance. These findings differ substantially from those observed for maintenance of CD4 T cells, suggesting fundamentally distinct roles of self-peptide–MHC ligands in regulating the two T cell subsets.

MATERIALS AND METHODS

Mice. WT C57BL/6 (B6; CD45.2, Thy1.2), B6.SJL (CD45.1), and B6.PL (Thy1.1) mice were obtained from the National Cancer Institute (Frederick, MD) or The Jackson Laboratory. H-2KdH/-/H-2DdH/- (KdD-/-) mice were originally purchased from Taconic Farms and intercrossed with B6.PL animals (The Jackson Laboratory) to produce Thy1.1 congenic KdD-/- mice. OT-I mice were intercrossed with B6.PL mice to generate Thy1.1, Hemozygous and Thy1.1/-1.2 heterozygous mice. KdD-/- Rag-/- OT-I TCR transgenic mice were originally obtained from E. Schott and H. Ploegh (National Institutes of Health, Bethesda, MD). All mouse strains were bred in vivo priming of OT-I cells. Host mice were injected i.v. with 3 × 106 WT OT-I or KdD-/- OT-I donor cells. DCs were enriched from the spleen cell pool obtained from normal WT mice using anti-CD11c magnetic beads as previously described (Fischer et al., 2007). Enriched DCs were pulsed with 10 nM OVAp (SINNEK) for 1 h at 37°C. After an intensive wash, 105 I-Ab+CD11c+ cells were injected i.v. into mice pretransferred with OT-I cells. Control mice were given unpulsed DCs. Spleens and lymph nodes were obtained from host mice 18 h following DC transfer, and the phenotype of CD8 cells reactive with Kd-OVAp tetramer was analyzed by flow cytometry.

In vitro activation assay. WT OT-I (Thy1.1) or CFSE-labeled KdD-/- OT-I cells were adoptively transferred at 3–5 × 106/mouse. Spleen cells or lymph node cells harvested from hosts placed in 96-well plate at 2 × 106/well with the half number of irradiated (2500 rads) WT spleen cells pulsed with various peptides, including B-Cat, G4, N6 (10 µM) or OVAp (10 nM; Hogquist et al., 1994; Jameson et al., 1993; Santori et al., 2002). For mixed stimulation experiments (Fig. 5, g and h), donor OT-I cells from different congenic markers (Thy1.1Thy1.2 and Thy1.1/Thy1.2) were transferred at 4–5 × 106/mouse. Lymph node cells from each group of hosts and irradiated WT splenocytes were mixed at equal number and placed in 48-well plate at 6 × 105/well with or without G4 (10 nM). In some experiments, freshly isolated WT naive OT-I cells were similarly stimulated and analyzed. The OT-I response was measured at 6 h (for CD69 and pc-Jun assays) or 48 h (for CFSE dye dilution assays) after initiation of the cultures. For CD8 blockade experiments, OT-I cells (from indicated sources) were cultured with anti-CD8α antibody (53–5.8; BD) or anti-CD8α antibody (53–6.7) at the indicated doses. After 30 min incubation (at room temperature), the OT-I cells were mixed with B6 splenocytes which were pulsed with G4 or OVAp peptides (or unpulsed). At 6 h, OT-I cells were stained for available CD8α and CD8β expression (determined by staining with fluorescent 53–5.8 or 53–6.7 antibodies) and CD69 induction.

MHC-I blocking in vitro. Spleen cells were cultured (105/ml) for 24 h in the presence of anti-Kd (y3) and anti-Dd (28–14.8) mAb (20 µg/ml). Actino- mycin D (5 µg/ml; Sigma-Aldrich) or cycloheximide (10 µg/ml; Sigma- Aldrich) was added at the initiation of the cultures where indicated. In some experiments, the cells were washed intensively and adoptively transferred (at 3 × 106/mouse) to intact B6 mice (n = 3/group). Donor cells were analyzed from lymph nodes harvested 48 h after transfer.

Flow cytometry. Single-cell suspensions were prepared from spleen and lymph nodes by mechanical disruption. Cells were resuspended in phosphate-buffered saline (pH 7.4) plus 1% fetal calf serum and incubated with fluorochrome-labeled mAbs. Antibodies with the following specificities were used for analysis: CD4 (L3T4), CD8α (53–6.7), CD8β (53–5.8; BD); B220 (RA3-6B2), CD5 (53–7.3), CD44 (IM7), CD45.1 (A20), CD26L (MEL-14), CD69 (H1.2F3), IL-7 receptor α (IL-7Rα; A7R34), TCR β (H57-597), and Thy1.1 (HHS1; eBioscience). TCR β Vβ repertoire analysis was performed using a TCR βV Screening Panel kit from BD. Biotinylated monomeric OVAp/Kd was prepared as previously described (Kao et al., 2005), and mixed with allophycocyanin-conjugated streptavidin (Invitrogen) at an 8:1 molar ratio (1:1 mass ratio), followed by incubation at room temperature for 1 h before staining. Staining of phospho-histidyl e-Jun followed the previously described protocol (Rosette et al., 2001), sequentially using purified anti-phospho-serine 63–c-Jun (KM1; Santa Cruz Biotechnology, Inc.), biotin-conjugated anti-mouse IgG1 (A85–1; BD), and streptavidin-phycocerythrin (BD). For intracellular staining of TNF, cells were stimulated with peptides in the presence of GolgiStop (BD). 6 h later, cells were treated with CytoTox/Cytoperm (BD) after surface staining, and were reacted with anti-TNF (MP6-XT22; BD) mAb. CFSE dye dilution was assessed in the FL-1 channel. Data were collected by FACSCalibur or LSR II instruments (BD) and analyzed on FlowJo software (Tree Star, Inc.).
Statistical analysis. Statistical significance was determined by unpaired or paired (for Fig. 5, g and b) Student’s t test with two-tailed distributions. P values <0.05 were considered significant. In all figures, one, two, and three asterisks indicate P < 0.05, 0.01, and 0.001, respectively. The survival data in Fig. 2c was also analyzed by ANOVA and by least squares means and multiple comparisons (using Tukey’s method), which confirmed the statistical significance of the comparisons discussed.

Online supplemental material. Fig. S1 shows the phenotype of WT donor cells after transfer to BM chimeric recipients that are partially or completely MHC-I−. Fig. S2 shows the phenotype of KbDb−/− donor cells after transferred to BM chimeric recipients that partially or completely lack MHC-I−. Fig. S3 shows the recovery of WT donor cells after short-term transfer into intact WT and MHC-I−deficient hosts. Fig. S4 shows that the enhanced reactivity of MHC-I−deprived CD8 T cells is restored by the treatment with low dose 53–6.7 antibody. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20082553/DC1.

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