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Cutting Edge: Differential Self-Peptide/MHC Requirement for Maintaining CD8 T Cell Function versus Homeostatic Proliferation

Ali Jabbari* and John T. Harty2*†

Memory T cells do not require self-peptide/MHC (spMHC) complexes to survive long term in vivo. However, memory CD4 T cells lose the ability to reject skin grafts when transiently placed in an environment in which these low-level TCR stimulations are absent. Whether or not spMHC alters the ability of CD8 T cells to respond to stimulation in vivo remains unknown. Here, we show that memory CD8 T cells retain the ability to respond to dendritic cell-mediated stimulation after adoptive transfer into either TAP+/− (MHC class I-deficient) or wild-type mice. Surprisingly, naive CD8 T cells, which fail to undergo homeostatic proliferation and erode in number in the absence of MHC class I, also retain the ability to respond to dendritic cell-mediated antigenic stimulation for at least 1 wk after transfer into TAP+/− mice. These findings suggest a differential requirement for spMHC signals for maintenance of CD8 T cell function and homeostatic proliferation. The Journal of Immunology, 2005, 175: 4829–4833.

Self-peptide/MHC (spMHC)3 class I complexes play a central role in T cell development in the thymus (1–3). Additionally, these spMHC class I signals affect T cell biology in the periphery. Naive CD4 and CD8 T cells require spMHC signals to undergo homeostatic proliferation in a lymphopenic environment (4–6). Naive CD8 T cells also require spMHC class I for long-term peripheral survival in an otherwise normal host (7, 8). Although previous studies indicated that naive CD4 T cells could survive independently of MHC class II (9), these experiments in Aβ−/− mice were potentially complicated by aberrant MHC class II dimer formation (10), and naive CD4 T cells may indeed require spMHC interactions for survival. Conversely, memory T cells have the ability to survive and undergo basal proliferation in the absence of spMHC signals (7, 11). These studies suggest a role for spMHC signals in the peripheral survival of at least some classes of naive, but not memory, T cells.

The requirements for spMHC for maintenance of T cell function has been most extensively explored for CD4 T cells. Studies report reduced (12) or enhanced (13) naive CD4 T cell responses in the absence of spMHC class II signals; these differences may depend on the circumstances of activation (14–16). Interestingly, memory CD4 T cells generated in spMHC-deficient hosts irreversibly lose the ability to mediate skin graft rejection in vivo (17). Together, these studies demonstrate a role for spMHC-TCR interactions in the survival and functional maintenance for at least some subsets of CD4 T cells.

The role of spMHC class I in the maintenance of CD8 T cell function remains undefined. Specifically, whether or not spMHC class I interactions with TCR are necessary to maintain memory CD8 T cell responsiveness to Ag in vivo has not been elucidated. In addition, it is not known whether spMHC class I signals necessary to induce homeostatic proliferation of naive CD8 T cells are necessary for the maintenance of CD8 T cell function.

Here, we established a model system whereby naive or memory OT-I TCR transgenic CD8 T cells were transferred into irradiated wild-type C57BL/6 (B6) mice or mice deficient in the TAP-1 gene (18). In TAP−/− mice, stable surface expression of MHC class Ia and MHC class Iβ molecules is severely compromised due to the lack of available peptides, and this minimal level of spMHC class I is not sufficient to induce homeostatic proliferation of naive CD8 T cells. After a rest, recipient mice were immunized with mature dendritic cells (DCs) from TAP−/− mice coated with the ova257–264 peptide, which binds to and stabilizes the surface expression of the presenting H2-Kb molecule. This system provides for both a host environment as well as an APC without substantial spMHC class I expression in which the Ag-specific CD8 T cell responses can be determined.

Materials and Methods

Mice and bacteria

B6 mice were purchased from the National Cancer Institute. TAP−/− mice (B6.129-S2-Tap1tm1Arp/J) were purchased from The Jackson Laboratory. OT-I (C57BL/6-Tcrb(Tg(Tcrb)1100MJb/f) mice (19) were provided by Dr. T. L. Ratliff (University of Iowa). Memory OT-I mice were generated by transferring 5 × 10⁶ naive OT-I (Thy1.1+ ) CD8 T cells, enriched by negative selection (StemCell Technologies), into naive B6 mice, followed by infection with 10⁶ attenuated (actD-deficient) Listeria monocytogenes expressing the OVA protein (20). Naive or memory (at least 60 days after infection) OT-I CD8 T cells were...
purified with PE-conjugated anti-Thy1.1 (BD Pharmingen) and anti-PE magnetic beads (Miltenyi Biotec) before transfer into irradiated (6.5 Gy; 24 h before transfer) recipients. In some cases, donor cells were labeled with 0.5 μM CFSE.

Bone marrow-derived DCs

DCs were generated from B6 or TAP−/− bone marrow and matured with LPS in vitro as described previously (21). DCs were coated with 1 μM peptide consisting of aa 257–264 of hen egg OVA (ova257–264) for 1 h and washed three times. A total of 5–7.5 × 10^5 DCs were injected i.v. per mouse.

Intracellular cytokine staining

Intracellular cytokine staining for IFN-γ was performed as described previously (22).

Results

TAP−/− cells are deficient in spMHC class I expression but can present exogenously added peptides

TAP−/− mice are unable to transport proteasomal degradation products into the endoplasmic reticulum, limiting the supply of self- or foreign-derived peptides and thereby preventing MHC class I complexes from being stably expressed on the cell surface (18). Initially, we determined whether the MHC class I-dependent process of naïve CD8 T cells to undergo homeostatic proliferation in lymphopenic hosts was abrogated in TAP−/− mice. We transferred CFSE-labeled, Thy1.1− naïve or memory OT-I TCR transgenic CD8 T cells into TAP−/− or B6 (Thy1.2+) mice. To prevent rejection of OT-I CD8 T cells and to generate a similarly lymphopenic environment, all recipients were sublethally irradiated before T cell transfer. As shown in Fig. 1A, naïve OT-I CD8 T cells underwent several divisions within 7 days after transfer into irradiated B6 mice but failed to divide over the same period in irradiated TAP−/− hosts. This supports previous studies of homeostatic proliferation (5) and is consistent with observations that wild-type CD8 T cells exhibit markedly decreased TCR ζ-chain tyrosine phosphorylation after transfer into TAP−/− hosts (23). Consistent with previous data (7), memory CD8 T cells underwent homeostatic proliferation in the TAP−/− hosts (Fig. 1B), albeit at a slower rate than in B6 hosts. These results confirm that MHC class I-dependent homeostatic proliferation by naïve CD8 T cells is defective in the TAP−/− host, demonstrating its suitability as a spMHC class I-low environment. Importantly, as seen in similar systems (7), the spMHC expression on the relatively low number of adoptively transferred T cells was insufficient to drive homeostatic proliferation.

Some in vitro studies suggest that T cell responsiveness to fully agonistic peptide is affected by spMHC on the surface of the APC (24), although this is not a universal finding (25). To avoid this potentially confounding variable, we used TAP−/−-derived DCs as APCs. Using the mAb 25-D1.16, which is specific for the Kb-ova257–264 peptide complex (26), we determined that bone marrow-derived, LPS-matured DCs from TAP−/− mice loaded with ova257–264 peptide presented similar amounts of Kb-ova257–264 complex as ova257–264 loaded B6-DCs (Fig. 1C). Additionally, binding of the ova257–264 peptide did not alter the total amount of MHC class I on the cell surface of either B6- or TAP−/−-DCs (data not shown). Thus, TAP−/−-DCs, despite being impaired in surface spMHC class I expression, can display exogenous ova257–264 peptide. Furthermore, TAP−/− and B6-DCs were similarly able to induce naïve OT-I T cell expansion in vivo (Fig. 1D), demonstrating no deficiency in the stimulatory potential of TAP−/−-DCs in the B6 host environment.

Robust memory CD8 T cell responses in a spMHC class I-low environment

Memory CD8 and CD4 T cells do not require spMHC class I or class II complexes for long-term survival (7, 11). However, recent studies suggest a possible role for spMHC in maintaining memory CD4 T cell function (17). To determine whether memory CD8 T cells also require spMHC class I signals in vivo to maintain function, we first transferred purified naïve, OT-I (Thy1.1) CD8 T cells to naïve, B6 (Thy1.2) hosts and infected recipients with attenuated Listeria monocytogenes expressing the OVA protein (20). Approximately 12 wk after infection, the memory OT-I CD8 T cells (>99% CD45high, ~65% CD62Lhigh; data not shown) was purified with anti-Thy1.1 Abs and transferred into irradiated B6 or TAP−/− hosts. Others have shown that partial TCR ζ-chain tyrosine phosphorylation declines only 15 min after T cell removal from a MHC-sufficient environment for CD4 cells (12) and by 2 days after transfer for CD8 T cells (23); to ensure that the transferred OT-I T cells had time to adapt to the MHC class I-low environment, we waited 7 days before immunizing these mice with ova257–264-coated TAP−/−-DCs. Seven days after immunization, memory OT-I cells expanded in the spleens of DC-immunized TAP−/− mice at least as well as in the B6 hosts in terms of both frequency (Fig. 2A) and total numbers (Fig. 2B), and both groups were capable of producing IFN-γ (see Fig. 4, right panels). Therefore, the
lack of substantial spMHC class I signals for up to 1 wk in vivo does not compromise memory OT-I T cell responses to DC-Ag stimulation.

Robust naive CD8 T cell responses in TAP−/− hosts

Naive CD8 T cells do not survive long term (5) nor undergo homeostatic proliferation in spMHC class I-deficient mice (Fig. 1A). Because the decline in numbers of naive T cells takes weeks to occur in spMHC-deficient mice (7), we were able to determine whether spMHC class I signals affected the ability of naive CD8 T cells to respond to DC-mediated antigenic stimulation in vivo. Purified, naive OT-I CD8 T cells were transferred into irradiated B6 or TAP−/− hosts. Because naive CD8 T cells undergo productive homeostatic proliferation in B6 but not TAP−/− hosts, we set up an additional group of irradiated B6 mice that received less naive OT-I CD8 T cells, such that their numbers in the spleen would be matched to those in TAP−/− hosts at the time of immunization. We then rested the mice for 7 days to ensure the T cells had adapted to the host environment. Longer rests were not used due to potential complications of repopulation of the immune compartment and rejection of the donor cells. All mice were immunized with TAP−/−-DCs coated with ova257–264 and the numbers of OT-I CD8 T cells in the spleens were assessed 7 days after immunization. Naive OT-I CD8 T cells underwent substantial expansion in response to antigenic stimulation in the TAP−/− host environment (Fig. 3, A and B) and were capable of producing IFN-γ (Fig. 4, left panels). Surprisingly, the response of the naive OT-I CD8 T cells in the TAP−/− mice appears to be greater than in the B6 hosts, as has been suggested by other in vitro studies (27). Importantly, T cells undergoing homeostatic proliferation in irradiated B6 hosts were able to expand in response to DC immunization just as well as T cells transferred into nonirradiated B6 hosts (data not shown), indicating that the act of homeostatic proliferation does not compromise proliferation in irradiated B6 mice. Therefore, the lack of substantial spMHC class I signals for up to 1 wk in vivo does not compromise naive CD8 T cell responses to DC-mediated antigenic stimulation.

Discussion

Here, we have shown that the levels of spMHC class I that are necessary for homeostatic proliferation of naive CD8 T cells are not required to maintain naive or memory CD8 T cell function. Our results contrast with some studies of CD4 T cells, in which spMHC is required for both homeostatic proliferation of naive cells (4) and to maintain function in both naive (12) and memory (17) CD4 T cells. This difference may reflect dissimilarities in CD4 and CD8 T cell biology or may be a consequence of differences between model systems. In regard to the latter possibility, although experiments in MHC class II-deficient hosts are not complicated by MHC class II expression on the transferred CD4 population, CD8 T cell transfer experiments may be confounded by spMHC class I expression on the transferred T cells. Studies examining the role of spMHC class I expression by APCs on CD8 T cell responses to DC-Ag stimulation may be confounded by spMHC class I expression on the transferred T cells. Studies examining the role of spMHC class I expression by APCs on CD8 T cell responses to DC-Ag stimulation.
cell activation in vitro (25) likewise may be complicated by the presentation of spMHC class I by the T cells to each other. In addition, spMHC class I expression by TAP \(^{-/-}\) cells, although severely impaired, is not completely absent. However, the low levels of spMHC class I on the surface of the host cells and the normal levels of spMHC class I on the rare, transferred T cells are insufficient to induce homeostatic proliferation of naïve CD8 T cells. We can therefore conclude that, in the absence of sufficient spMHC class I signals to induce naïve CD8 T cell homeostatic proliferation, naïve and memory CD8 T cells retain the ability to mount a vigorous response to Ag. This, at the least, defines a difference in the threshold of spMHC class I expression necessary to induce homeostatic proliferation and to maintain function. The undiminished, and perhaps amplified, response of naïve CD8 T cells in the spMHC class I-deficient host suggests, however, that CD8 T cells do not require spMHC class I-mediated stimulation to remain functional; additional studies examining whether and the extent to which NK cells may affect the CD8 T cell response to TAP \(^{-/-}\)-DCs are underway, although our data indicate no difference in the stimulatory capacity of TAP \(^{-/-}\)-DCs compared with B6-DCs in irradiated B6 mice (Fig. 1D).

On the other hand, these results contrast with data illustrating that CD4 T cells exposed to diminished levels of spMHC class II exhibit decreased responsiveness in vitro and in vivo (12, 17, 28) (although this is not universally accepted (13)) and may be reflective of biological differences between CD8 and CD4 T cells. Although they fill different roles in the immune system, the requirements of naïve CD4 and CD8 T cell activation for foreign peptide/MHC and costimulation, provided by mature DCs in secondary lymphoid organs, are fundamentally similar. Why, then, would naïve CD4 and CD8 T cells have different requirements for spMHC interactions to maintain function? One possible explanation relates to the location of where T cells interact with spMHC. Naïve T cells circulate between the blood and secondary lymphoid organs. Along this route, naïve CD4 T cells will be able to interact with spMHC class II, which is restricted to DCs, B cells, and macrophages, most frequently in secondary lymphoid organs. Therefore, naïve CD4 T cell interactions with spMHC will mainly occur within the environment in which productive encounters with mature DC-expressing agonist peptide-MHC complexes will initiate an immune response. In this way, the sensitivity to antigenic stimulation of naïve CD4 T cells could be enhanced by encounter with spMHC in secondary lymphoid organs and decreased when naïve CD4 T cells are in the circulation. In contrast, MHC class I is expressed on essentially all nucleated cells, and thus naïve CD8 T cells likely undergo continual interactions with spMHC class I complexes. Under these circumstances, a requirement for spMHC class I to maintain responsiveness to Ag could not be used to limit initial CD8 T cell priming to secondary lymphoid organs.

In contrast to naïve T cells, memory T cells are able enter peripheral tissues with relative ease. Memory CD4 T cells therefore will be able to recognize spMHC class II at sites of inflammation where DCs, macrophages, and B cells accumulate and ectopic MHC class II expression may occur, in addition to secondary lymphoid organs. Thus, the responsiveness of CD4 T cells to activation may be enhanced by interaction with spMHC class II at these sites. In turn, this may facilitate CD4 T cell coordination of DCs, macrophages, and B cells to respond to the immunological input. Regulating memory CD4 T cell responses such that they are enhanced in secondary lymphoid organs and peripheral sites of inflammation and reduced at other sites may therefore be a mechanism of ensuring potent responses at appropriate sites and preventing memory CD4 T cell activation at inappropriate sites. Memory CD8 T cells, in contrast, directly interact with infected cells in peripheral tissues. Because expression of spMHC class I is ubiquitous throughout the body, its ability to affect memory CD8 T cell function would not be enhanced or reduced in any location. Alteration of memory CD8 T cell responsiveness with respect to spMHC class I distribution therefore would not be an effective mechanism at limiting responses to appropriate locations.

Here, we have shown that there is a lower requirement for spMHC class I levels in the maintenance of CD8 T cell responsiveness vs the induction of homeostatic proliferation of naïve CD8 T cells. One potential explanation for this differential requirement may lie in the context of when these phenomena occur. Homeostatic proliferation in naïve T cells occurs in a relatively empty immunological compartment, in which the number of T cells competing for binding to spMHC class I ligands is decreased. The amount of spMHC class I that any individual T cell sees therefore may be greater in such an environment when compared with a full immunological compartment in which homeostatic proliferation does not need to occur. The ability to respond to pathogenic infections, however, needs to be able to occur in a full immunological compartment in which more competition is present for spMHC class I ligands. From this perspective, it seems logical that maintenance of CD8 T cell function requires less dependence on spMHC class I signals than on homeostatic proliferation.

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Disclosures
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