A Low Affinity TCR Ligand Restores Positive Selection of CD8+ T Cells In Vivo

Heather E. Stefanski, Dita Mayerova, Stephen C. Jameson and Kristin A. Hogquist

J Immunol 2001; 166:6602-6607; doi: 10.4049/jimmunol.166.11.6602
http://www.jimmunol.org/content/166/11/6602

References
This article cites 37 articles, 19 of which you can access for free at:
http://www.jimmunol.org/content/166/11/6602.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
A Low Affinity TCR Ligand Restores Positive Selection of CD8\(^+\) T Cells In Vivo\(^1\)

Heather E. Stefanski, Dita Mayerova, Stephen C. Jameson, and Kristin A. Hogquist\(^2\)

The T cell repertoire is shaped by the processes of positive and negative selection. During development, the TCR binds self peptide-MHC complexes in the thymus, and the kinetics of this interaction are thought to determine the thymocyte’s fate. For development of CD8\(^+\) T cells, the data supporting such a model have been obtained using fetal thymic organ culture. To confirm the fidelity of this model in vivo, we studied development of OT-I TCR-transgenic mice that expressed different individual K\(^b\) binding peptides in thymic epithelial cells under the control of the human keratin 14 promoter. We used a system that allowed TAP-independent expression of the peptide-MHC complex, such that the ability of given peptides to restore positive selection in TAP\(^-\) mice could be assessed. We found that transgenic expression of a TCR antagonist peptide (E1) in vivo efficiently restored positive selection of OT-I T cells in TAP\(^-\) mice. An unrelated transgenic peptide (SIY) did not restore selection of OT-I T cells, nor did the E1-transgenic peptide restore selection of an unrelated receptor (2C), showing that positive selection is peptide specific in vivo, as observed in organ cultures. Neither E1 nor SIY transgenes increased the polyclonal CD8 T cell repertoire size in non-TCR-transgenic animals, arguing that single class I binding peptides do not detectably affect the size of the CD8 T cell repertoire when expressed at low levels. We also observed that OT-I T cells selected in TAP\(^-\)-E1 mice were functional in their response to Ag; however, there was a lag in this response, suggesting that the affinity of the TCR interaction with MHC-self peptide can result in fine-tuning of the T cell response. The Journal of Immunology, 2001, 166: 6602–6607.

During positive selection in the thymus, only precursor cells bearing TCRs that are self-MHC-restricted are allowed to develop. The TCR recognizes a combinatorial ligand of self peptide and MHC. It has therefore been of interest to understand how self peptides influence the selection of MHC-restricted precursors, given that the TCR does not recognize MHC molecules independently of associated self peptide. Initial experiments involving fetal thymus organ cultures (FTOC)\(^3\) showed that specific peptides promoted positive selection of individual class I MHC-restricted CD8\(^+\) T cells (reviewed in Ref. 1). These data argue that greater peptide diversity would select a more diverse T cell repertoire. Recently, in vivo experiments using elegant strategies to modify the peptide content of class II MHC molecules contested this idea. Analysis of mice theoretically expressing a single peptide-MHC II complex showed selection of a large population of CD4\(^+\) T cells (2–4). T cells from such mice could recognize a broad range of peptide Ags regardless of whether they were structurally related to the selecting ligand (3–6). This was taken as evidence that positive selection of many T cells is peptide independent or peptide promiscuous. However, further analysis showed that less diverse TCR V-J joins were used in T cells from single-complex mice (7). Additionally, recent experiments have questioned whether the dominant peptide is actually the mediator of positive selection or whether the few endogenous peptides present at extremely low levels in these mice are more important (8, 9).

The model system we used to explore the peptide specificity of thymic selection uses the OT-I TCR-transgenic mouse. The OT-I TCR recognizes the chicken OVA 257–264 peptide, SIINFEKL (OVA), in the context of K\(^b\). Initial experiments using FTOC showed that the peptide E1INFEKL (E1), an antagonist variant of the OVA peptide, promoted positive selection of OT-I cells, whereas OVA itself caused deletion (10, 11). The half-life of OT-I binding to E1-K\(^b\) was subsequently found to be 44 times shorter than that to OVApeptide\(^-\) when measured at 37°C (12). If organ cultures appropriately mimic in vivo development, these data strongly suggest that kinetic discrimination is the basis for the differential response of thymocytes during positive and negative selection. However, the development of CD8 T cells in FTOC has been noted under conditions that would not be permissive for CD8 development in vivo (13), and it has been suggested that CD8 T cells might develop directly from DN precursors in organ cultures, bypassing the double-positive (DP) stage of development (14, 15). Furthermore, in two cases where peptides that were identified as positive selection ligands using FTOC were expressed in vivo as transgenes, positive selection was not observed (16) (and M. Bevan, unpublished observations).

For these reasons, it was important to determine whether the E1 low affinity ligand could promote positive selection of OT-I thymocytes in vivo when presented endogenously by cortical epithelial cells. In this study we created a transgenic mouse expressing the E1 peptide using the human keratin 14 (K14) promoter, which has been shown to allow expression in cortical epithelial cells (17). We found that the presence of E1 in vivo efficiently restored positive selection of OT-I T cells, but did not increase the size of the CD8 repertoire in non-TCR-transgenic mice. The implications of these observations are discussed.
Materials and Methods

Peptide-transgenic construction

The adenosvirus E19/3K signal sequence-peptide expressing construct was generated using a multistep PCR procedure to produce insert oligomers coding for the adenosvirus signal sequence (MRYMLGGLLALAVCSAA) and the E1 peptide (EINFKEKL) or the SIY peptide (SIYRRYYGL). The PCRs were performed with three oligomers: O1 (5′-GGG AAG CTT AGC ATG ATA TAC ATG CGC GGC CTG CTG-3′) with a HindIII site at the 5′ terminus; O2 (5′-GGC CTG CTG GCC CTG GCC GGC GTG TGC AGC GCC GCC-3′) with a BsteII site and 2 nucleotides at the 3′ terminus; and O3 for E1 (5′-TTT CTC GAG TCA CAG CTT CCA GTA GAT CAT CTC GAC GGC GCT-3′) or O3 for SIY (5′-TTT CTC GAG TCA CAG CTT GCC GTA GTC GCT GGC GCT-3′), with an XhoI site at the 5′ terminus. The terminal base pairs of O2 were complementary to O3 and permitted their joining through PCR. Similarly, the terminal base pairs of O1 were complementary to O2, permitting construction of the desired insert, including sequences from O1 to O2. The O2 and O3 oligomers were initially joined using a 50-μM PCR consisting of PCR buffer (10× assay buffer buffer B; Fisher, Pittsburgh, PA), 2.5 mM MgCl2, 300 μM dNTPs, and oligomers at 20 μM/mL. The cycle conditions were 95°C for 30 s, 30°C for 60 s, and 72°C for 30 s for a total of 30 cycles. This was followed by a second PCR in which the O1 and O3 oligomers were added to 5 μL of the first PCR. The second reaction consisted of 10 cycles as described above, followed by 30 cycles of 95°C for 1 min and 72°C for 2 min. The resultant products were then digested with HindIII and XhoI and directly cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA), and the orientation and fidelity of the cloning were confirmed by sequence analysis.

The K14 promoter construct was a gift from E. Fuchs (University of Chicago, Chicago, IL). A number of modifications to the K14 vector were performed before inserting the SS peptide, including conversion of the HindIII (position 4770) site to a KpnI and creation of HindIII and XhoI sites at the BamHI site (position 2150). Sequence analysis confirmed the fidelity of the cloning. The excised construct was sent to The Jackson Laboratory (Bar Harbor, ME) or University of Minnesota Cancer Center for microinjection into C57BL/6 embryos. Three founders were obtained for K14-SS-E1, and two were obtained for K14-SS-SIY.

The following primers were used in a PCR to determine whether the mice were transgenic for K14-SS-peptide: forward, 5′-CCC TCT ATT GTA TGC GCC ATT-3′, and reverse, 5′-TCTGAAACTAGGACC CATT-3′. These mice were bred to the OT-I TCR or 2C TCR-transgenic mice on an H-2b wild-type or TAP-deficient background.

Flow cytometry

Four-color staining was performed using FITC-labeled anti-B20.1 (the transgenic Vo2 chain), PE-labeled anti-CD4 (BD Pharmingen, San Diego, CA), CyChrome-labeled anti-CD8 (BD Pharmingen), and allophycocyanin-labeled anti-CD8a (BD Pharmingen), followed by TriColor or CyChrome-labeled streptavidin (BD Pharmingen), and allophycocyanin-labeled anti-CD8b (BD Pharmingen). The cells were analyzed on a FACS Calibur (BD Biosciences, Mountain View, CA) using CellQuest software.

CD69 up-regulation assay

Splenocytes from a C57BL/6 TAP-deficient mouse were cultured with or without peptide for 1 h at room temperature. Unbound peptide was washed away, and the cells were plated at a concentration of 5 × 106 cells/well in a round-bottom 96-well plate. Lymph node cells or splenocytes were excised from the different OT-I strains and placed at 5 × 105 cells/well. The cells were spun together at 1000 rpm for 5 min and then placed at 37°C for 3 or 24 h. The cells were stained with FITC-anti-CD69, PE-anti-CD4, and allophycocyanin-anti-CD8 (BD Pharmingen). At least 1000 CD8 events were collected and analyzed using a FACS Calibur with CellQuest software (BD Biosciences).

Proliferation assay

Lymph node cells from OT-I or OT-I-TAP-E1 mice were suspended at a concentration of 1–5 × 106 cells/ml in HBSS. After warming the cells at 37°C, they were stained with CFSE (Molecular Probes, Eugene, OR) for 10 min at 37°C with occasional mixing. After 10 min ice-cold RPMI 1640 was added, and the cells were recovered by centrifugation. The cells were incubated with TAP* splenocytes pulsed in the presence or the absence of 100 nM OVAp for 44 h at 37°C. At this time the cells were stained with biotin anti-CD69, followed by allophycocyanin-labeled anti-CD8-a and streptavidin-PerCP. At least 10,000 CD8 events were collected and analyzed using a FACS Calibur with CellQuest software (BD Biosciences).

CTL assay

Lymph node cells from wild-type OT-I or OT-I-TAP+E1 mice were stimulated in the presence of OVAp in vitro for 5 days. The cells were harvested and used as effectors in a chromium release assay with EL4 target cells as previously described (10).

Results and Discussion

A transgenic construct for the presentation of class I binding peptides in the thymus in a TAP-independent manner

To test whether the low affinity ligand, E1, could promote positive selection in OT-I mice in vivo, we created peptide-transgenic mice. We chose a strategy that would direct loading of the peptide into MHC molecules and presentation at the cell surface in a TAP-independent manner. This allowed us to ask whether positive selection, which is impaired in TAP-deficient animals, could be restored by peptide in vivo. The transgene construct encoded a fusion protein consisting of an endoplasmic reticulum (ER) signal sequence followed by the eight amino acids of the peptide. Fusion proteins of a similar nature have been shown to target peptides to the ER in a TAP-independent manner in vitro (18) and in vivo (16, 19). Once in the ER, the peptide is cleaved, presumably by signal peptidase, and is presented in the context of MHC class I molecules at the cell surface (18). We also created transgenic mice expressing the Kβ binding peptide SIYRRYYGL (SIY) as a control (20). Transient transfection of the fusion protein constructs into tumor cells confirmed that the peptides were presented by Kβ to T cells in a TAP-independent manner (data not shown).

The fusion protein was expressed under control of the human K14 promoter (17, 21). K14 is expressed in epithelial cells in the thymus and in basal cells of the skin and esophagus. Specifically, we were interested in obtaining expression in epithelial cells of the thymic cortex, the microenvironment in which positive selection occurs (22). The human K14 promoter was shown to reproducibly direct expression of transgenes in thymic cortical epithelial cells (17, 23, 24) (I. Williams and I. Frazier, unpublished observations).

An E1 transgene restores positive selection of OT-I cells in vivo

It was previously observed that E1 peptide induced positive selection of CD8+ T cells in OT-I β-μ-microglobulin” mice (10, 11) and OT-I TAP+ mice (15) in FTOC. To test whether this peptide could induce positive selection of OT-I cells in vivo, we crossed the K14-E1 strain to OT-I and backcrossed to the TAP+ background. The thymus, spleen, and lymph node cells were compared with those of OT-I-TAP+ (a control for positive selection) and OT-I-TAP- (a control for no selection). Fig. 1 shows cell surface levels of CD4, CD8, and the OT-I-transgenic α-chain (Vo2). The E1 transgene efficiently restored positive selection of CD8 single-positive (CD8SP) thymocytes in OT-I-TAP+ mice. There was a dramatic increase in both the level of TCR on total thymocytes and the percentage and number of TCRhigh CD8SP (Fig. 1A). Additionally, E1-transgenic mice showed a distinct population of CD4+ CD8dim cells that were absent in OT-I-TAP+ mice. This population was also quite prominent in the OT-I wild-type (OT-I) thymus and was previously shown to be a transitional population between DP precursors and CD8 single-positive mature cells (25). The apparent increase in CD4+ CD8- cells (5 to 11%) between OT-I-TAP+ and OT-I-TAP- E1 mice does not reflect an increase in positive selection to the CD4 lineage. In fact, the absolute number of mature (heat-stable Ag low) CD4+ thymocytes was 2- to 6-fold lower in OT-I-TAP+E1 mice compared with OT-I-TAP+ mice. This decrease in CD4 selection is reflected in the spleen as well (Fig. 1B).

Recently, Levelt et al. studied the development of a different class I-restricted TCR-transgenic (F5) using a similar approach...
A LOW AFFINITY LIGAND RESTORES POSITIVE SELECTION IN VIVO

The presence of E1 in vivo restores positive selection of OT-I T cells. Thymocytes (A) or splenocytes (B) from OT-I-TAP<sup>−</sup>, OT-I-TAP<sup>−</sup>-E1, or OT-I mice were stained with Abs to CD4, CD8, and V<sub>o2</sub> and analyzed by FACS. The numbers indicate the percentages of TCR<sup>high</sup> cells. The histograms show TCR levels on total thymocytes, and the number shown is the percentage of TCR<sup>high</sup> cells in the gate. The plots are representative of >12 mice analyzed from two different founders.

(16). They introduced the NP34 peptide preceded by a signal sequence as a transgene in mice and studied the development of CD8 cells expressing the F<sub>5</sub> TCR (16). In FTOC, this peptide was shown to induce positive selection of F<sub>5</sub> T cells (26), but in vivo it did not do so (16). Goldrath et al. reported an analogous approach using the K<sup>b</sup> promoter to drive expression of an OVAp variant (R4) (19), which had been shown to induce positive selection of OT-I T cells in vitro (10), but did not restore positive selection in vivo (M. Bevan, unpublished observations). At this point, we do not understand the nature of these discrepancies. Although the promoters used by these groups directed expression in a variety of tissues as judged by Northern blot analysis (16), there was no information about which cells in the thymus expressed it. Thus, it is possible that the peptide expression in cortical epithelial cells was not sufficient to support positive selection. It is important to note here that while we used a cortical epithelial cell-specific promoter, we were unable to directly demonstrate that the E1 peptide was processed and presented by K<sup>b</sup> in these cells (data not shown). We tested this by measuring OT-I thymocyte CD69 up-regulation after exposure to purified cortical epithelial cells in vitro. However, it is likely that this assay was not sensitive enough to detect the low affinity E1 ligand, because no thymic APC (bone marrow-derived or stromal) from E1-transgenic mice showed activity in this assay, and a high level of exogenous E1 peptide caused only modest up-regulation of CD69 when presented by cortical epithelial cells. Additionally, thymic stromal cells from transgenic mice expressing the high affinity OVAp ligand in the same expression vector (23) were strongly positive in this assay. Thus, given the strong effect of the E1 transgene on positive selection in vivo, it seems likely that the lack of in vitro activity from E1-transgenic stromal cells was due to assay insensitivity and not to a lack of tissue-specific expression from this promoter. Nonetheless, it is difficult to comment on whether differences in peptide expression led to the disparate results in the above model systems. Levelt et al. also showed evidence that the NP34 peptide antagonized positive selection in TAP<sup>−</sup><sup>−</sup> F<sub>5</sub> animals (16). Therefore, it might be possible that a broad expression of peptide Ag (on both bone marrow-derived cells and epithelial cells) results in inhibition of positive selection, while exclusive expression on epithelial cells promotes it. However, we believe this unlikely, because inhibition of positive selection was not observed in FTOC in which exogenous peptide was also broadly presented, nor did Goldrath and colleagues observe antagonism of positive selection with R4 expressed by the K<sup>b</sup> promoter (M. Bevan, unpublished observations).

The positive effect of the K14-E1 transgene on selection of CD8 T cells in OT-I-TAP<sup>−</sup> mice was also reflected in spleen and lymph node populations (Fig. 1B). The percentage and number of V<sub>o2</sub> T cells are increased in OT-I-TAP<sup>−</sup>-E1 compared with those in OT-I-TAP<sup>−</sup> mice. Surprisingly, the extent of restoration appeared somewhat smaller in the periphery than in the thymus (Fig. 1, compare A with B). Typically, the reverse situation is seen, where positive selection in the thymus is impaired, but large numbers of peripheral cells can accumulate (27). The observation that the K14-E1 transgene efficiently promoted positive selection in the thymus but did not result in a large peripheral population may reflect an unavailability of the E1 peptide in the periphery to sustain naive T cell survival. Recent data suggest that mature T cell survival may require recognition of self MHC molecules on dendritic cells (28). The potential cross-presentation of skin Ags by dendritic cells in lymphoid organs may not be efficient enough in the absence of TAP<sup>−</sup> to support naive T cell survival in the OT-I-TAP<sup>−</sup>-E1 mouse strain. This possibility is currently under investigation.

**Positive selection of CD8<sup>+</sup> T cells is peptide specific**

The fact that selection of OT-I thymocytes was restored suggests that the E1 peptide was present at an appropriate level and in the proper thymic compartment to influence positive selection. However, it was possible that any K<sup>b</sup> binding peptide expressed by this promoter could restore positive selection by stabilizing K<sup>b</sup> molecules at the surface of cortical epithelial cells. Thus, we examined mice expressing the peptide SIYRYYG (SIY) under the control of the K14 promoter. This peptide is a target Ag for the 2C TCR-transgenic. Thus, we analyzed 2CxK14-SIY mice to confirm the in vivo expression of the SIY peptide. In 2CxK14-SIY mice, the 2C TCR clonotype-positive DP cells were reduced in number by 50-fold in the presence of K14-SIY (data not shown), confirming thymic expression of the Ag in the cortex. To determine whether positive selection is peptide specific, we crossed K14-SIY mice with OT-I-TAP<sup>−</sup>-TAP-transgenic mice. We found that positive selection of OT-I T cells was not restored in these animals (Fig. 2A) as it was by K14-E1. To further test whether peptide specificity is important in positive selection, we crossed the 2C TCR-transgenic mice to the K14-E1 strain. As shown in Fig. 2B, positive selection in 2C-TAP<sup>−</sup> mice was not restored by K14-E1 as it was in OT-I-TAP<sup>−</sup> mice. Finally, to test whether the addition of a single peptide in TAP<sup>−</sup> mice would restore selection of CD8 T cells, we looked at the CD8 compartment in TAP<sup>−</sup>-E1 mice. We found that the K14-E1 transgene had no detectable effect on the percentage or number of polyclonal CD8 T cells in non-TCR-transgenic TAP<sup>−</sup> mice (Fig. 2C). Together, these data confirm the FTOC data, suggesting that positive selection is a peptide-specific process and that individual peptides do not support the selection of a large fraction of the repertoire when expressed at low levels. Although the E1 peptide may have caused subtle alterations in the repertoire in non-TCR-transgenic mice, it did not have a large effect on the size of the CD8 compartment. This is in contrast to the increase in CD8 (29) or CD4 T cells (2, 3, 5) observed in other models in which the MHC-self peptide diversity is restricted to a single complex. Unlike those other experimental systems, we did not observe an up-regulation of cell surface MHC levels in E1-TAP<sup>−</sup> cells in vitro, and this mouse probably did not have high levels in vivo (data not shown).
cytes from 2C wt, 2C-TAP o, or 2C-TAP o-E1 were stained with Abs to CD4, of cells in the gate indicated are 21, 1, and 1% (left to right). B, Thymocytes from 2C wt, 2C-TAP o, or 2C-TAP o-E1 were stained with Abs to CD4, CD8, and the clonotypic TCR Ab 1B2 and analyzed by FACS. The percentages of cells in the gate indicated are 29, 2, and 2% (left to right). C, B6, TAP o, or TAP o-E1 mice were stained with Abs to CD4 and CD8 and analyzed by FACS. The percentages of cells in the gate indicated are 4.2, 0.1, and 0.1% (left to right).

**FIGURE 2.** Positive selection of CD8⁺ T cells is peptide specific. A, Thymocytes from OT-I wt, OT-I-TAP o, or OT-I-TAP o-SIY were stained with Abs to CD4, CD8, and Vα2 and analyzed by FACS. The percentages of cells in the gate indicated are 21, 1, and 1% (left to right). B, Thymocytes from 2C wt, 2C-TAP o, or 2C-TAP o-E1 were stained with Abs to CD4, CD8, and the clonotypic TCR Ab 1B2 and analyzed by FACS. The percentages of cells in the gate indicated are 29, 2, and 2% (left to right). C, B6, TAP o, or TAP o-E1 mice were stained with Abs to CD4 and CD8 and analyzed by FACS. The percentages of cells in the gate indicated are 4.2, 0.1, and 0.1% (left to right).

**FIGURE 3.** OT-I T cells selected in K14-E1-TAP o mice are functional. We wanted to determine whether the cells that matured in OT-I-K14-E1-TAP o mice were functional. We tested lymph node cells for their ability to respond to the antigenic peptide or to low affinity ligands tested (Fig. 3, A and B). At 3 h CD8 T cells from OT-I-TAP o-E1 mice had a 50% lower surface expression of CD69 compared with normal OT-I CD8 T cells. However, by 24 h both populations expressed equivalent levels of CD69 in response to all ligands (Fig. 3A). Interestingly, this delay was not reflected by a shift in the dose-response curve to OVAp even at low doses or at early time points (Fig. 3B) or to a shift in the dose response to ligands of lower affinity (data not shown). Moreover, there was no difference between the two populations in proliferation, as assessed by a CFSE dye dilution assay at 48 h (Fig. 3C). The similarity between the populations was observed at 36, 48, and 60 h and at various peptide doses (not shown). We also generated CTL lines from OT-I and OT-I-TAP o-E1 spleen cells and observed that their cytolytic activity against peptide-coated targets was equivalent (Fig. 4), although we acknowledge that the in vitro culture may have selected for the most highly reactive responders. Taken together, these data suggest that although there is a slight lag in the response of CD8 T cells from OT-I-TAP o-E1 mice, it is not reflected in decreased sensitivity and does not hinder them from making a functional response to Ag, at least in vitro.

We were interested in determining whether there were differences between OT-I T cells that matured in the presence of E1 compared with those matured in the normal environment that could account for this lag in response to Ag. The TCR α- and β-chains were expressed at similar levels between the two populations (Fig. 4, A and B). More importantly, OVAp tetramer bound both cell types to a similar level (Fig. 4C). Although there was no difference in CD8α expression, there was a decrease in CD8β expression in cells from OT-I-TAP o-E1 mice (Fig. 4, D and E). There was also a slightly higher CD5 level in CD8 T cells from OT-I-TAP o-E1 mice compared with wild-type OT-I T cells (Fig. 4F).

The lag in response might be due to the slight differences in CD5 and CD8β expression levels on cells from OT-I-TAP o-E1 mice. Lower coreceptor and increased CD5 levels have been noted in TCR transgenics expressing agonist or stimulatory ligands in vitro and in vivo (11, 30–32). Here we note the effect of a low affinity variants of it. Interestingly, there was a delay by CD8 T cells from OT-I-TAP o-E1 mice in the up-regulation of CD69 in response to all ligands (Fig. 3A). Interestingly, there was a delay by CD8 T cells from OT-I-TAP o-E1 mice in the up-regulation of CD69 in response to all ligands (Fig. 3A). Interestingly, this delay was not reflected by a shift in the dose-response curve to OVAp even at low doses or at early time points (Fig. 3B) or to a shift in the dose response to ligands of lower affinity (data not shown). Moreover, there was no difference between the two populations in proliferation, as assessed by a CFSE dye dilution assay at 48 h (Fig. 3C). The similarity between the populations was observed at 36, 48, and 60 h and at various peptide doses (not shown). We also generated CTL lines from OT-I and OT-I-TAP o-E1 spleen cells and observed that their cytolytic activity against peptide-coated targets was equivalent (Fig. 4), although we acknowledge that the in vitro culture may have selected for the most highly reactive responders. Taken together, these data suggest that although there is a slight lag in the response of CD8 T cells from OT-I-TAP o-E1 mice, it is not reflected in decreased sensitivity and does not hinder them from making a functional response to Ag, at least in vitro.

We were interested in determining whether there were differences between OT-I T cells that matured in the presence of E1 compared with those matured in the normal environment that could account for this lag in response to Ag. The TCR α- and β-chains were expressed at similar levels between the two populations (Fig. 4, A and B). More importantly, OVAp tetramer bound both cell types to a similar level (Fig. 4C). Although there was no difference in CD8α expression, there was a decrease in CD8β expression in cells from OT-I-TAP o-E1 mice (Fig. 4, D and E). There was also a slightly higher CD5 level in CD8 T cells from OT-I-TAP o-E1 mice compared with wild-type OT-I T cells (Fig. 4F).

The lag in response might be due to the slight differences in CD5 and CD8β expression levels on cells from OT-I-TAP o-E1 mice. Lower coreceptor and increased CD5 levels have been noted in TCR transgenics expressing agonist or stimulatory ligands in vitro and in vivo (11, 30–32). Here we note the effect of a low affinity
variant that promoted positive selection in the thymus. These data suggest that the presence of E1 in vivo forces a molecular compensation in developing thymocytes that adjusts their response through the TCR. Alternatively, the effect might be achieved not by receptor-ligand-driven changes in the cell, but by selection of only those cells that had a predetermined cell surface level of these molecules. However, if this were the case, we would expect to see a large decrease in cell number in the thymus of OT-I-TAP\textsuperscript{o}-E1 mice, which we did not. The CD8\textsuperscript{b} and CD8\textsuperscript{a} data argue more for a conversion than a selection, because virtually all preselection DP thymocytes express a high level of CD8\textsuperscript{b} and a low level of CD8\textsuperscript{a}. However, upon selection OT-I-TAP\textsuperscript{o}-E1 thymocytes have uniformly lower levels of CD8\textsuperscript{b} and higher levels of CD8 than OT-I\textsuperscript{m} thymocytes. In addition, in vitro systems have shown that CD8 levels may be directly down-regulated to modify the response (33). Recently, Love and coworkers examined the level of CD5 in a number of different TCR-transgenic systems (32). Their studies showed that positive selection up-regulates CD5 levels and that CD5 is even further up-regulated under negative selection conditions. CD5 was shown to be a negative regulator of cell signaling (34, 35). Taken together these data provide a model in which modulation of the TCR signal by CD5 to enhance selection can be envisioned. Our data support this model. However, it has been suggested that CD5 and CD8\textsuperscript{b} do so by altering the activation threshold (13, 30). In an experimental system using a lymphocytic choriomeningitis virus-specific TCR, it was shown that the spectrum of activating ligands for a given mature TCR is altered depending on the ligands that are present in the thymus during selection (36). Another experimental system has supported the model of T cell tuning in development. The 2C TCR is normally positively selected on K\textsuperscript{b}, but is alloreactive to L\textsuperscript{d}. When 2C TCR-transgenic T cells were positively selected by low levels of H-2L\textsuperscript{d}, they were unable to respond to endogenous levels of the self peptide p2Ca-L\textsuperscript{d} complexes (37). However, addition of exogenous p2Ca could induce a T cell response. This suggests that the 2C thymocytes were modified so that a response to the selecting ligand activated mature T cells. In our system the slight differences in CD5 and CD8\textsuperscript{b} receptor levels correlate with a delayed response, rather than an altered threshold. Our study has shown that the presence of E1 in vivo is unable to restore positive selection of a diverse TCR repertoire in TAP\textsuperscript{a} mice, but is able to restore positive selection of functional OT-I CD8\textsuperscript{b} T cells. These data support a model in which immature thymocytes are positively selected and modified by interactions with discrete self peptides.

Acknowledgments

We thank W. C. Kieper, B. Krause, C. Liquori, B. Koehn, and D. Erlandson for technical assistance, and members of the Hogquist and Jameson laboratories for critically reviewing the manuscript.

References

14. Bruno, L., H. J. Fehling, and H. von Boehmer. 1996. The a\textbeta T cell receptor can replace the \gamma\delta receptor in the development of \gamma\delta lineage cells. Immunity 5:543.
28. Lundberg, K., W. Heath, F. Kontgen, F. R. Carbone, and K. Shortman. 1995. Intermediate steps in positive selection: differentiation of CD4\textsuperscript{+} TCR\textsuperscript{m} thymocytes into CD4\textsuperscript{+} TCR\textsuperscript{hi} thymocytes. J. Exp. Med. 181:1643.


