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Designing and Maintaining the Mature TCR Repertoire: the Continuum of Self-Peptide:Self-MHC Complex Recognition

Christophe Viret, F. Susan Wong, and Charles A. Janeway, Jr.*
Section of Immunobiology
Yale University School of Medicine
and Howard Hughes Medical Institute
New Haven, Connecticut 06520-8011

Summary

Peripheral T cell maintenance requires a survival signal delivered upon T cell receptor (TCR)-major histocompatibility complex (MHC) molecule interaction. Since self-peptides play a critical role in the intrathymic positive selection of the mature TCR repertoire, we hypothesized an equally important role in T cell persistence. We used mice with a normal expression of MHC class II molecules but a restricted self-peptide complexity (H-2M$\alpha^{-/-}$) to show that an MHC class II-restricted T cell specificity that displays a deficient positive selection in the H-2M$\alpha^{-/-}$ thymus shows an impaired persistence after adoptive transfer in H-2M$\alpha^{-/-}$ recipients. Finally, a wild-type CD4$^+$ TCR repertoire is incompletely maintained in H-2M$\alpha^{-/-}$ recipients. These observations suggest that, similar to intrathymic positive selection, the maintenance of the mature TCR repertoire relies on the recognition of self-peptide:self-MHC complexes.

Introduction

Mature $\alpha\beta$ T lymphocytes (CD3^{hi} CD4$^+$CD8$^-$ or CD4$^-$ CD8$^+$) are generated in the thymus via developmental processes consisting of positive (Robey and Fowlkes, 1994; von Boehmer, 1994; Kisielow and von Boehmer, 1995) and negative selection (Nossal, 1994) and exported to the periphery where they constitute the naive pool of recirculating T cells able to react to foreign peptide antigens. Previous experimental work with CD8$^+$ T cells has suggested an important role for self-peptides in positive selection of thymocytes and in shaping the mature TCR repertoire (Nikolic-Zugic and Bevan, 1990; Sha et al., 1990; Ashton-Rickardt et al., 1993; Hogquist et al., 1993; Jameson et al., 1995). This concept of "peptide-specific positive selection" recently received support from in vivo observations made with single TCR β chain Tg mice in which the self-peptide complexity has been shown to have a direct impact on the diversity of the mature TCR repertoire of CD4$^+$ T cells: the limited self-complexity of the thymic environment leads to positive selection of a restricted TCR repertoire (Sant'Angelo et al., 1997).

The TCR repertoire shaped in the thymus can be further conditioned in the periphery. For instance, peripheral clonal deletion can take place to maintain tolerance to extrathymic self-determinants (Webb et al., 1990; Rocha and von Boehmer, 1991), and the size of the peripheral T cell pool is maintained by homeostatic control. Studies using CD8$^+$ transgenic T cells revealed that the emergence of newly generated T cells (thymus migrants) leads to substitutions in the peripheral pool of naive and tolerant T cells (possibly by competition for the same niches) but not in the pool of memory T cells that appears to be controlled by a distinct homeostatic regulation (Tanchot and Rocha, 1995, 1997). However, which fine mechanisms are involved in the maintenance of the mature T lymphocytes is still incompletely understood. Adoptive transfer experiments have shown that most naive T cells can persist in SCID recipients (Sprent et al., 1991) and that transgenic naive CD8$^+$ T cells specific for the male antigen (HY) can survive for long periods in the absence of antigenic stimulation since the number remains stable in female nude recipients (von Boehmer and Hafen, 1993; Bruno et al., 1995). The requirement of a signal received through the TCR upon MHC interaction for the survival of both mature CD8$^+$ and CD4$^+$ T cells was documented recently using grafts of fetal thymus as well as adoptive transfer of T cells (Takeda et al., 1996; Kirberg et al., 1997; Rooke et al., 1997; Tanchot et al., 1997). Remarkably, a signal delivered through the B cell receptor appears to be required for the maintenance of mature B lymphocytes as well (Lam et al., 1997), suggesting that the receipt of a survival signal through clonotypic immune receptors is a general principle in the biology of lymphocytes involved in adaptive immunity (Neuberger, 1997).

The accumulating observations that indicate a central role for self-peptides in the shaping of the TCR repertoire (Viret and Janeway, 1999) may suggest an equally important role for self-peptide:self-MHC complexes in many aspects of the T cell physiology, including in the full activation of T lymphocytes when recognition of foreign peptides occurs and also in the maintenance of the peripheral T cell pool (Janeway et al., 1998). Experiments reported so far do not address the question of how important the self-peptide repertoire is in the delivery of the survival signal to peripheral T cells when the MHC-TCR interaction occurs. To investigate this aspect, we took advantage of the H-2M-deficient (H-2M$\alpha^{-/-}$) mice (Fung-Leung et al., 1996; Martin et al., 1996; Miyazaki et al., 1996). These mice lack the alpha subunit of the H-2M$\alpha\beta$ heterodimer that is critical for the loading of antigenic peptides into the MHC class II molecules in endosomal/lysosomal compartments (Denzin and Cresswell, 1995). APCs from the H-2M$\alpha^{-/-}$ mice dominantly express the invariant chain (Ii)-derived 81-104 peptide (the CLIP peptide) bound to I-A^b. This is reminiscent of studies related to mutant cell lines (Riberdy et al., 1992; Sette et al., 1992). However, H-2M$\alpha^{-/-}$ APCs also express some other endogenous peptides at a low level since there is evidence that two immune receptors unable to interact with the CLIP peptide:I-A^b complex do interact with MHC class II positive cells from the H-2M$\alpha^{-/-}$ mice (Grubin et al., 1997); the BP107 mAb can inhibit positive selection of H-2M$\alpha^{-/-}$ CD4$^+$ T cells in

*To whom correspondence should be addressed (e-mail: charles.janeway@yale.edu).

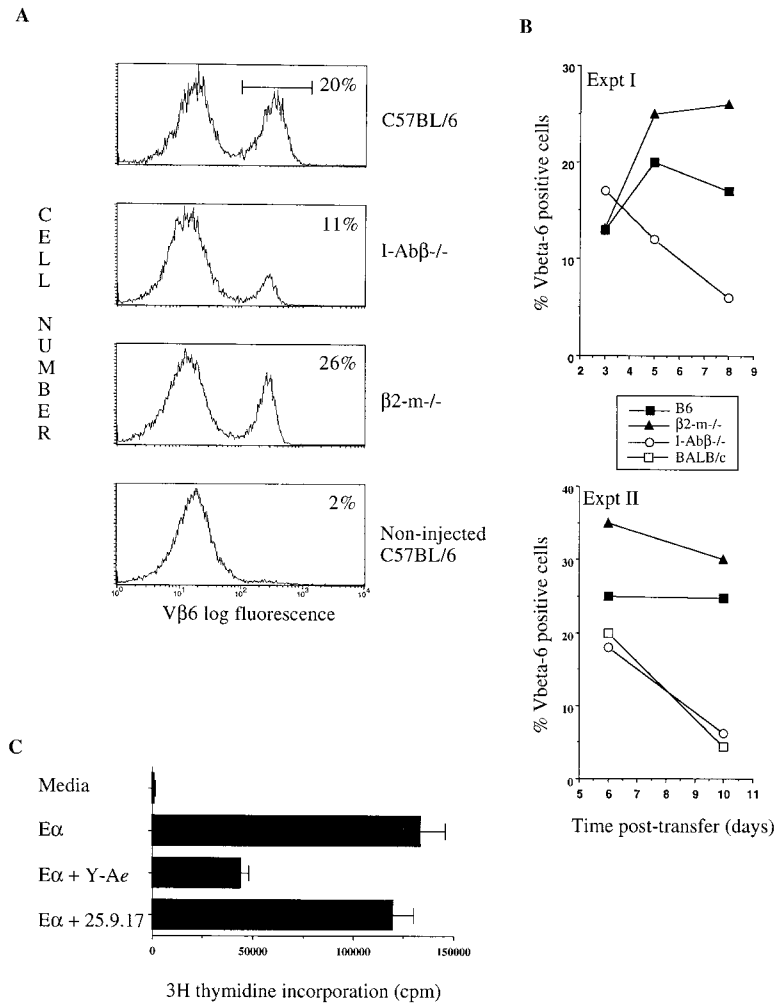

Figure 1. Differential Persistence of Adoptively Transferred Purified 1H3.1 TCR Tg CD4$^+$ T Cells in Secondary Lymphoid Organs of Thymectomized-Irradiated Wild-Type and Mutant Recipient Mice

(A) An example of FACS analysis of a Vβ6 staining performed on ammonium chloride–treated spleen cell suspensions 5 days after intravenous injection of 6×10^6 Tg CD4$^+$ T cells. The bottom histogram shows splenocytes from a noninjected control mouse.

(B) Maintenance of mature naive Tg CD4$^+$ T cells requires the surface expression of the restricting MHC class II molecules. Plots represent the percentage of recovered Vβ6$^+$ cells from spleen as shown in (A). Similar results were obtained with lymph node–derived cells. Experiments where the various recipient mice were concomitantly analyzed are shown. Consistent data were obtained when mutant mice were tested independently together with normal B6 mice. Representative absolute numbers of recovered Vβ6$^+$ splenocytes in B6, I-A^{b}β$^{-/-}$, β2m$^{-/-}$, and BALB/c mice were, respectively, 0.3–0.4 $\times$ 10^6 at day 3 for all recipients, 0.64 $\times$ 10^6, 0.08 $\times$ 10^6, 0.67 $\times$ 10^6, and 0.02 $\times$ 10^6 at day 5, and 2.1 $\times$ 10^6, 0.09 $\times$ 10^6, 2.35 $\times$ 10^6, and 0.03 $\times$ 10^6 at day 8.

(C) Transgenic T cells recovered from the spleen of a thymectomized-irradiated B6 recipient at day 10 post transfer retain the ability to react specifically to the cognate peptide in vitro. Lymph node cells from recipients were stimulated in the presence of irradiated B6 APCs and 5 μg/ml Eα52-68 (Ea). The Y-Ae and 25.9.17 mAbs were used at 1 μg/ml. Data are representative of two experiments.

fetal thymic organ culture (FTOC) experiments and a T cell hybrid specific for a beta-2 microglobulin (β2m)-derived peptide bound to I-A^b can react significantly to H-2Mα$^{-/-}$splenocytes. Thus, the H-2Mα$^{-/-}$ mice display a restricted self-peptide complexity rather than a unique peptide/I-A^b complex.

In this study, we use a TCR transgenic-based experimental system to dissect the requirement for MHC class II molecule expression in the delivery of survival signals to mature CD4$^+$ T cells in the periphery. We then address the question of whether the self-peptides presented by MHC class II molecules directly contribute to the maintenance of mature T cells as they do in the shaping of the mature TCR repertoire via intrathymic positive selection of immature thymocytes.

Results

Peripheral Persistence of Purified Mature TCR Tg CD4 T Cells in Thymectomized-Irradiated Recipients Requires the Expression of the Positively Selecting MHC Class II Molecules

Hypothesizing that self-peptide:self-MHC complexes may have as important a role in the maintenance of the peripheral T cell pool as they have in the shaping of the mature TCR repertoire via intrathymic positive selection of immature thymocytes (Janeway et al., 1998), we performed adoptive transfer of transgenic CD4$^+$ T cells and followed their persistence using flow cytometry. Mature CD4$^+$Vβ6$^+$ T cells were purified from spleen and lymph nodes of 6- to 8-week-old Tg mice expressing the 1H3.1 TCR (Vα1-Vβ6) that is specific for the Eα52-68 peptide presented in the context of the I-Aαβ^b MHC class II heterodimer (Rudensky et al., 1991a, 1991b). Antigen-presenting cell (APC)-depleted Tg 1H3.1 CD4$^+$ T cells were intravenously injected into various thymectomized-irradiated recipient mice including C57BL/6 (B6), B6-β2 microglobulin-deficient mice (β2m$^{-/-}$) lacking surface expression of most MHC class I molecules, B6-MHC class II–deficient mice (I-A^{b}β$^{-/-}$), and BALB/c mice that express a distinct MHC haplotype (H-2^d). At different time points, spleen and lymph node cell suspensions were prepared, and the fraction of persisting transgenic T cells was determined by Vβ6 staining. The results shown in Figures 1A–1B indicate that persistence of Tg T cells seen in the B6 animals requires the expression of MHC class II molecules on APCs of the recipient mice. This is demonstrated by comparing the maintenance of transferred cells in wild-type B6 mice versus their decline in the MHC class II–deficient recipients. Figure 1A

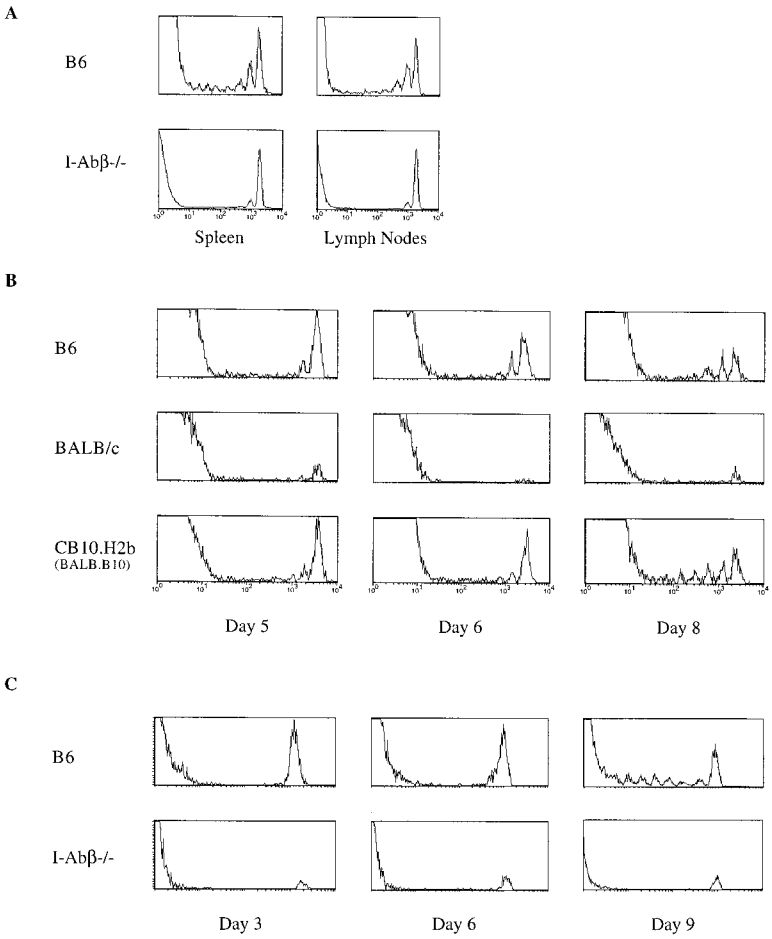

Figure 2. MHC Class II Molecule-Dependent Division of 1H3.1 TCR Tg CD4$^+$ T Cells in Secondary Lymphoid Organs of Irradiated Syngeneic Recipient Mice

(A) CFSE-labeled 1H3.1 TCR Tg CD4$^+$ T cells undergo cell division after transfer into normal syngeneic mice (B6) but virtually none when injected into mice lacking expression of I-A^b molecules (I-A$^b\beta^{-/-}$). Cell suspensions were prepared from the spleen and lymph nodes of recipients at day 9 after adoptive transfer and analyzed by cytofluorometry.

(B) Cytofluorometric analysis of cells recovered from the spleen of B6 (H-2^b), BALB/c (H-2^d), and CB10.H-2^b (BALB.B10) mice at different time points after adoptive transfer of CFSE-labeled 1H3.1 TCR Tg CD4$^+$ T cells.

(C) Cytofluorometric analysis of cells recovered from the spleen of normal B6 and MHC class II–deficient (I-A$^b\beta^{-/-}$) mice at various time points after transfer of CFSE-labeled 1H3.1 TCR Tg CD4$^+$ RAG-1$^{-/-}$ T cells. Comparable profiles were observed for lymph node cells.

shows a detailed example of FACS analysis and includes a control animal that received no cells. At day 3, the spleen of wild-type and mutant recipient mice contain a comparable number of Vβ6$^+$ T cells that then differentially evolve depending on the host MHC genotype (see Figure 1B legend). The differential persistence was usually clear at day 4–5 post transfer. Since it was reported that naive CD8$^+$ T cells require the positively selecting MHC class I molecule to persist after adoptive transfer (Tanchot et al., 1997), we asked whether the same rule applies to our system. APCs from BALB/c mice, which do not cause proliferation of 1H3.1 TCR Tg T cells in an MLR reaction (data not shown), express I-E$\alpha\beta^d$ and I-A$\alpha\beta^d$ heterodimers that are structurally close enough to I-A$\alpha\beta^b$ to be able to bind the Eα52-68 peptide recognized by the 1H3.1 $\alpha\beta$ TCR (Hunt et al., 1992). We therefore used irradiated-thymectomized BALB/c as recipient mice. The result in Figure 1B (experiment II) shows that MHC class II I-A$\alpha\beta$ heterodimers distinct from the restricting element (I-A$\alpha\beta^b$) lead to an impaired persistence of transferred 1H3.1 TCR Tg T cells. Importantly, we checked that the transgenic T cells recovered from B6 recipient animals after 8–10 days are functionally competent. Vβ6$^+$CD4$^+$ T cells isolated from spleen or lymph nodes do react to the cognate peptide in vitro, indicating that they not only physically persist in vivo after transfer but effectively retain the ability to respond to antigen (Figure 1C). The specificity of the response is established by blocking experiments done using monoclonal antibodies (mAb); the I-A^b:Eα52-68 complex-specific mAb Y-A*e* (Murphy et al., 1992) can inhibit reactivity of 1H3.1 TCR Tg T cells, whereas the 25.9.17 mAb, which reacts to multiple I-A^b:peptide complexes but not to the I-A^b:Eα52-68 epitope (Chervonsky et al., 1998), has no effect.

Transferred CD4$^+$ Mature Lymphocytes Undergo Cell Division that Is Dependent on the Expression of the Restricting MHC Class II Molecules

In the normal B6 recipients, the fraction and number of recovered Vβ6$^+$ T cells were repeatedly lower at day 2 or 3 after transfer when compared to later time points. This could reflect either the requirement for a certain delay in the repopulation of the irradiated host lymphoid organs by the transferred cells or the occurrence of a certain level of cell division after transfer. To discriminate between these two possibilities, we labeled the purified 1H3.1 Tg CD4$^+$ T cells with the cytoplasmic dye CFSE prior to transfer into recipient mice. The cytometric analysis of CFSE-labeled cells allows the detection of cell division since the dye intensity is reduced when cells undergo mitosis (Lyons and Parish, 1994). Thus, freshly CFSE-labeled cells appear as a unimodal distribution that is converted to a multimodal distribution upon occurrence of mitosis. The cell cycling state was analyzed after recovery of transferred cells from recipient mice.

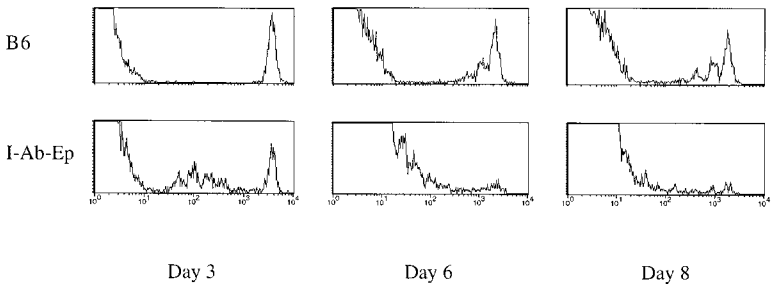

Figure 3. Moderate Cell Division of 1H3.1 TCR Tg CD4$^+$ T Cells after Adoptive Transfer into Irradiated Normal Syngeneic (B6) Recipient Mice

CFSE-labeled 1H3.1 TCR Tg CD4$^+$ T cells divide rapidly in irradiated recipient mice expressing the I-A^b:Eα52-68 complex at a low level on all APCs (I-A^b-Ep mice). Cell suspensions were prepared from the spleen of B6 and I-A^b-Ep recipients at day 3, 6, and 9 after adoptive transfer, ammonium chloride treated, and analyzed by cytofluorometry. Note the clear multimodal distribution observed at day 3 post transfer in the I-A^b-Ep recipient.

Figure 2A shows histograms of CFSE fluorescence after cell recovery at day 9 from normal B6 and MHC class II–deficient recipient mice. Cell division is clearly detected in the normal B6 recipient but is very marginal in the recipient mouse lacking expression of the selecting I-A^b MHC class II molecules. This indicates that transferred Vβ6$^+$CD4$^+$ T cells undergo cell division after transfer into a normal mouse and that such division is dependent on a TCR-MHC class II molecule interaction. The same type of experiment was performed using B6 versus BALB/c versus CB10.H-2^b (BALB.B10) congenic mice. Figure 2B shows that transferred T cells divide equally when transferred into B6 and CB10 H-2^b recipients (top and bottom panels) but not into the BALB/c recipient where the CFSE signal is barely detectable (middle panels). This demonstrates that the inability of 1H3.1 TCR Tg CD4$^+$ T cells to divide in the BALB/c lymphoid organs, as well as their disappearance, is a consequence of the lack of expression of the restricting MHC class II molecules (I-A^b) and not the result of a rejection mediated by residual host CD8$^+$ T cells able to recognize antigenic structures, such as minor histocompatibility antigens, on the donor cells. Finally, Figure 2C shows that division of the transferred 1H3.1 Tg CD4$^+$ T cells occurs also in normal B6 recipient mice when donor cells are isolated from 1H3.1 TCR Tg RAG-deficient (RAG-1$^{-/-}$) donor mice. This clearly demonstrates that the cell division we observe in the normal B6 recipient does not result from activation of dual TCR T cells potentially able to react to epitopes such as determinants derived from environmental antigens.

The MHC Class II–Dependent Cell Division of Transferred 1H3.1 TCR Tg CD4$^+$ T Cells in Normal B6 Recipients Corresponds to a Low Level of Division

In order to estimate the magnitude of the 1H3.1 Tg T cell division we observe in the normal B6 recipients after adoptive transfer, we performed a comparative analysis of transferred 1H3.1 TCR Tg CD4$^+$ T cells into normal B6 mice versus I-A^b-Ep mice (I-A$^b\beta^{-/-}$, Ii$^{-/-}$, I-A$^b\beta$-Eα52-68 Tg$^+$) (Ignatowicz et al., 1996) that exclusively express the I-A^b:Eα52-68 complex on all MHC class II positive cells, however with a reduced intensity due to the necessary absence of the invariant chain (Ii). As previously, Tg T cells were CFSE labeled prior to transfer, and the cycling status was analyzed using flow cytometry after cell recovery from spleen at different time points. Figure 3 shows that, in contrast with the B6 recipients, 1H3.1 Tg CD4$^+$ T cells have divided approximately six times in 3 days in the spleen of recipient mice expressing the I-A^b:Eα52-68 complex at a low level. At day 8, virtually all the CFSE-labeled cells have undergone division in the I-A^b-Ep mice. As a consequence, the I-A^b-Ep spleen was found to be enlarged and the number of Vβ6$^+$ T cells was strongly increased (data not shown). Thus, it appears that the level of the I-A^b-dependent cell division associated with the maintenance of the transferred cells into the normal B6 recipient is, both in terms of magnitude and kinetics, clearly distinct from the proliferation induced in vivo by recognition of the cognate peptide in the context of I-A^b on the surface of professional APCs.

Deficient Intrathymic Positive Selection of the 1H3.1 T Cell Specificity in the Presence of a Limited Self-Peptide Repertoire

To test the hypothesis that intrathymic positive selection of thymocytes and maintenance of mature T cells in the periphery involve a similar signal delivered by self-peptide:self-MHC complexes, we again used the 1H3.1 TCR Tg mice in experiments involving the H-2M$\alpha^{-/-}$ mice that, as indicated above, express a restricted I-Aβ:self-peptide complex repertoire dominated by the CLIP peptide. Very importantly, analysis done with various anti-I-A^b mAbs has shown that the expression level of MHC class II molecules on H-2M$\alpha^{-/-}$ APCs is identical to the level detected on H-2M$\alpha^{+/+}$ APCs (Fung-Leung et al., 1996; Martin et al., 1996; Miyazaki et al., 1996). It is demonstrated by staining with the Y3JP mAb that reacts to I-A^b in a peptide-independent manner. Our hypothesis predicts that if 1H3.1 TCR Tg thymocytes are positively selected on a restricted intrathymic self-peptide repertoire (the H-2M$\alpha^{-/-}$ thymic environment), mature 1H3.1 TCR Tg CD4$^+$ T cells should behave identically after adoptive transfer into B6 and H-2M$\alpha^{-/-}$ animals. Conversely, if positive selection is deficient on the H-2M$\alpha^{-/-}$ background, then we expect to observe a distinct result upon transfer into B6 and H-2M$\alpha^{-/-}$ recipients. The question of intrathymic positive selection was addressed by breeding. Results from the 1H3.1 TCR Tg $\times$ H-2M$\alpha^{-/-}$ cross (Figure 4A) show that the positive selection of immature 1H3.1 Tg thymocytes is clearly altered by the absence of H-2M: compared to TCR Tg H-2Mα24$^{+/-}$ thymus (top panels), a higher number of Vβ6^{high}CD4$^+$CD8$^+$ (double positive [DP]) thymocytes appears in the TCR Tg H-2M$\alpha^{-/-}$ thymus (TCR Tg, 1.46 $\times$ 10^6; TCR Tg H-2M$\alpha^{-/-}$, 70.94 $\times$ 10^6), and fewer

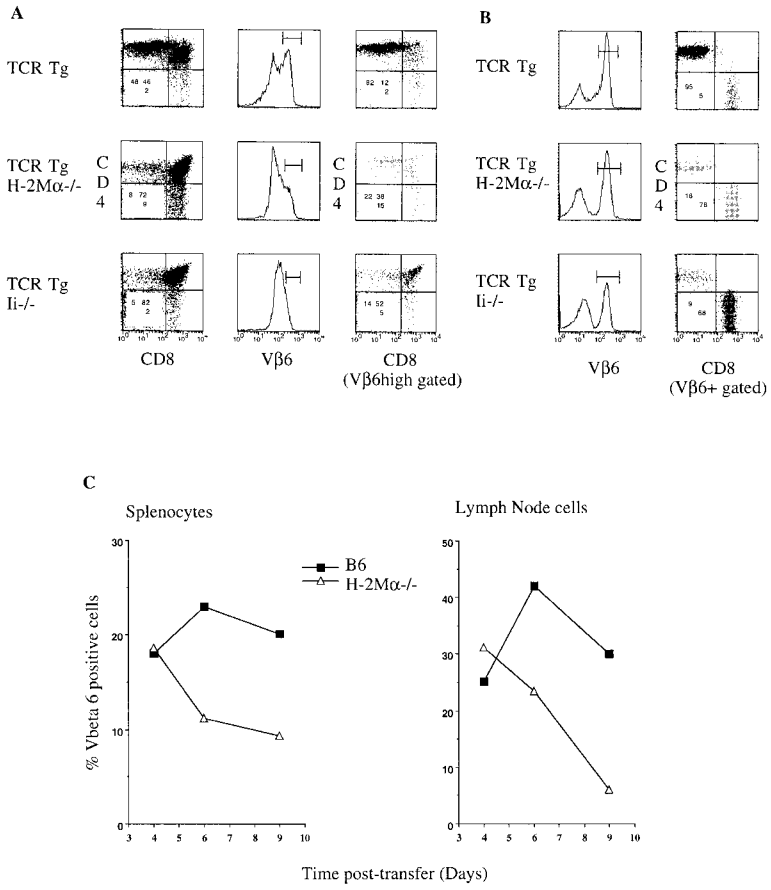

Figure 4. Deficient Positive Selection of the 1H3.1 Specificity in the H-2M$\alpha^{-/-}$ Thymic Microenvironment and Impaired Persistence of Mature 1H3.1 Tg T Cells upon Transfer to H-2M$\alpha^{-/-}$ Recipients

(A) FACS analysis of thymic cell suspensions prepared from 6- to 7-week-old 1H3.1 TCR Tg H-2M$\alpha^{+/-}$ and H-2M$\alpha^{-/-}$ littermate mice. The analysis of an 8-week-old 1H3.1 TCR Tg Ii$^{-/-}$ thymus is shown as a nonselecting situation for comparison. The central panels show the Vβ6 histogram. The left and right panels show the CD4/CD8 distribution, respectively, without and with electronic gating on the V$\beta6^{high}$ thymocyte population. Quadrant statistics are indicated. In the experiment depicted, the cellularity was: TCR Tg H-2M$\alpha^{+/-}$, 146 $\times$ 10^6; TCR Tg H-2M$\alpha^{-/-}$, 186.7 $\times$ 10^6; and TCR Tg Ii$^{-/-}$, 124.5 $\times$ 10^6.

(B) FACS analysis of lymph node cell suspensions. The right panels indicate the CD4/CD8 distribution after electronic gating on the V$\beta6^+$ cells. Thymic and lymph node profiles are representative of three mice analyzed.

(C) Deficient persistence of the adoptively transferred mature 1H3.1 TCR Tg CD4$^+$ T cells into irradiated H-2M$\alpha^{-/-}$ recipients. Plots represent the fraction of V$\beta6^+$ cells recovered from secondary lymphoid organs of B6 and H-2M$\alpha^{-/-}$ recipient mice after immunostaining and FACS analysis of cell suspensions at different time points after adoptive transfer. The fraction of V$\beta6^+$ cells in a noninjected irradiated B6 control mouse was 2.7% at day 9 (data not shown). The data are representative of three experiments. Left, splenocytes analysis; right, pooled lymph node cells analysis. Representative absolute numbers of CD4$^+$V$\beta6^+$ splenocytes at day 2, 4, and 8 were, respectively, 0.11 $\times$ 10^6, 0.42 $\times$ 10^6, and 1.28 $\times$ 10^6 for B6 recipients and 0.16 $\times$ 10^6, 0.04 $\times$ 10^6, and 0.08 $\times$ 10^6 for H-2M$\alpha^{-/-}$ recipients.

V$\beta6^{high}$CD4$^+$CD8$^-$ thymocytes are detected (middle panels) (TCR Tg, 119.7 $\times$ 10^6; TCR Tg H-2M$\alpha^{-/-}$, 41 $\times$ 10^6). In accordance, few V$\beta6^{high}$ thymocytes are detectable in the thymus (central histogram). The marginal positive selection is further reduced in the thymus of 1H3.1 TCR Tg/Ii$^{-/-}$ mice (bottom panels) that have a low MHC class II expression level. Such results may even reflect positive selection of thymocytes coexpressing alternate TCRs made of endogenously rearranged TCR α chains. The cellularity observed for the TCR Tg H-2M$\alpha^{-/-}$ thymus was repeatedly higher than for the TCR Tg H-2M$\alpha^{+/-}$ thymus (see Figure 4 legend). In the periphery, some V$\beta6^+$ T cells do accumulate in the TCR Tg H-2M$\alpha^{-/-}$ lymph nodes (Figure 4B, middle panels), but the vast majority express the CD8 coreceptor.

Impaired Peripheral Maintenance of an MHC Class II–Restricted TCR Specificity Confronted with a Restricted Self-Peptide Complexity

To address the question of the maintenance of the mature 1H3.1 TCR Tg T cells in the H2-M$\alpha^{-/-}$ peripheral environment, we then performed adoptive transfer of purified naive 1H3.1 TCR Tg CD4$^+$ T cells into irradiated B6 versus H-2M$\alpha^{-/-}$ mice and, as previously, looked for persistence in spleen and lymph nodes at different time points after transfer. In accordance with the prediction, the mature naive 1H3.1 TCR Tg T cells transferred into H-2M$\alpha^{-/-}$ recipient mice revealed a significantly impaired persistence compared to normal recipient mice (Figure 4C), indicating that the appropriate survival signal is not efficiently delivered by H-2M$\alpha^{-/-}$ APCs. This effect was clearly detectable at day 6–7 post transfer both in spleen and lymph nodes. Identical experiments were performed using CFSE-labeled cells. Figure 5 shows that the persistence of the transferred Tg cells in the B6 recipient (see Figure 4C) correlates, as mentioned above, with a low level of cell division. Conversely, the impaired persistence of the transferred cells seen in the periphery of the H-2M$\alpha^{-/-}$ mice correlates with a very marginal cell division. In addition, the cytometric pattern obtained for the H-2M$\alpha^{-/-}$ recipient (bottom panel) is very comparable to the pattern obtained for the MHC class II–deficient recipient (middle panels). This indicates that, for the 1H3.1 T cell specificity, the peripheral confrontation of a self-peptide repertoire unable to drive intrathymic positive selection is not significantly different from the total absence of TCR-MHC class II molecule interaction and therefore indicates that the peripheral

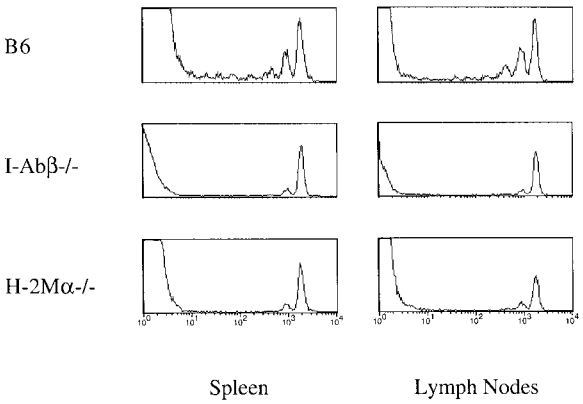

Figure 5. The Impaired Persistence of Adoptively Transferred 1H3.1 TCR Tg CD4$^+$ T Cells into the H-2M$\alpha^{-/-}$ Recipients Correlates with the Absence of Cell Division

Cell suspensions were prepared from the spleen and lymph nodes of normal B6, MHC class II–deficient (I-A^{b}b$^{-/-}$), and H-2M$\alpha^{-/-}$ recipients at day 9 after adoptive transfer of CFSE-labeled cells and analyzed by cytofluorometry.

persistence of mature T cells relies on a low division rate controlled by the specific recognition of self-peptides in secondary lymphoid organs.

Differential Behavior of Mature CD4$^+$ T Cells from H-2M-Deficient Mice after Transfer into Normal, H-2M-Deficient, and MHC Class II–Deficient Mice

Since the low level of cell division of mature Tg CD4$^+$ T cells was found to be self-peptide specific, we thought to analyze the behavior of mature CD4$^+$ T cells that have been positively selected on H-2M$\alpha^{-/-}$ thymic epithelial cells (i.e., mainly on the CLIP peptide:I-A^b complex). Transfer experiments reveal that these cells are able to divide in the H-2M$\alpha^{-/-}$ recipients but virtually not in the recipients lacking MHC class II molecules (Figure 6). These profiles are in sharp contrast with the high level of expansion observed in the B6 recipients, which is expected since peripheral H-2M$\alpha^{-/-}$ CD4$^+$ T cells react strongly to APCs from syngeneic wild-type mice (Fung-Leung et al., 1996; Martin et al., 1996; Miyazaki et al., 1996).

Persistence of Polyclonal CD4$^+$ but Not CD8$^+$ Mature T Cells Is Impaired in the Periphery of Recipient Mice Expressing a Restricted Self-Peptide: MHC Class II Complex Repertoire

We sought to also examine the behavior of CD4$^+$ T cells from nonmanipulated mice into H-2M$\alpha^{-/-}$ versus wild-type recipients. If a self-peptide independent TCR/MHC class II interaction, or even a nonspecific CD4/MHC class II interaction, is the only basis for peripheral persistence of mature CD4$^+$ naive T cells, no significant difference should be observed between the two recipients. Conversely, if self-peptides are involved in the survival signal delivered to T cells, the TCR repertoire that has developed in the thymus of a normal mouse should reduce its diversity (i.e., a significant fraction of cells should not be able to divide). To test this, we transferred

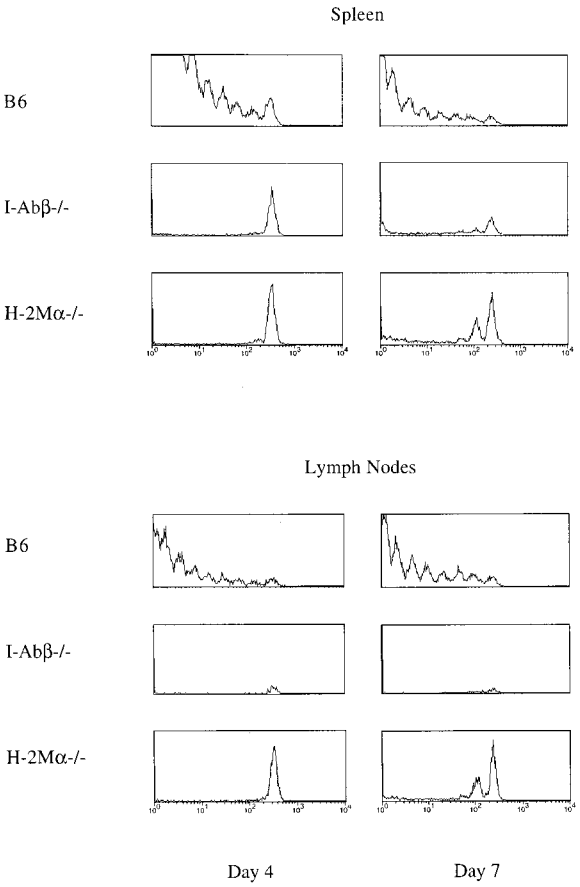

Figure 6. Differential Evolution of Mature CD4$^+$ T Cells from H-2M$\alpha^{-/-}$ Mice after Transfer into B6, H-2M$\alpha^{-/-}$, and I-A^{b}b$^{-/-}$ Mice

CFSE-labeled H-2M$\alpha^{-/-}$ CD4$^+$ T cells (5×10^6) were intravenously injected into irradiated recipients, and secondary lymphoid organs were analyzed at day 4 and 7 by flow cytometry. Histograms were plotted after electronic gating on CD4$^+$ T cells. The cell numbers ([cellularity $\times$ % CFSE$^+$ cells]/100) were, at day 4 and 7, respectively, 0.06×10^6 and 0.14×10^6 for the H-2M$\alpha^{-/-}$ recipients, 0.02×10^6 and 0.022×10^6 for the I-A$^b\beta^{-/-}$ recipients, and 0.36×10^6 and 0.35×10^6 for the B6 recipients. In the latter case, cell numbers are underestimated since many dividing cells reached the background fluorescence level.

purified CD4$^+$ T cells from spleen and lymph node of normal B6.PL mice (H-2^b, Thy-1.1) into irradiated B6 and H-2M$\alpha^{-/-}$ mice (both H-2^b, Thy-1.2). Figure 7A shows a difference in the absolute number of recovered CD4$^+$ T cells in the peripheral lymphoid organs of the H-2M$\alpha^{-/-}$ recipient mice, presumably reflecting the inability to divide by lack of signal from an appropriate self-epitope in the mutant mice. In accordance with this, CFSE labeling showed a clear difference in the division pattern (Figure 7B). In addition, when the experiment was concomitantly conducted with purified polyclonal CD8$^+$ mature T cells from B6.PL mice, no obvious change was observed in the absolute number of CD8$^+$ T cells recovered from the recipients (Figure 7C). This observation establishes that the inability of some CD4$^+$ T cells to divide and persist in the H-2M$\alpha^{-/-}$ mutant does not result from a rejection phenomenon.

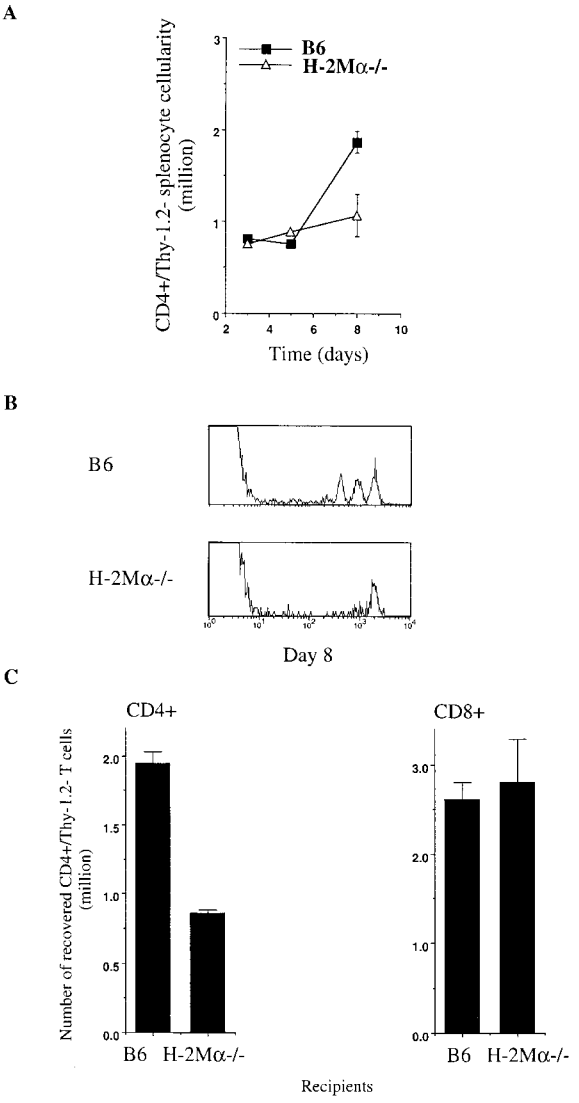

Figure 7. Impaired Maintenance of Adoptively Transferred Mature Polyclonal $CD4^+$ (but Not $CD8^+$) T Cells from Normal B6.PL (Thy-1.1^+) Mice into Irradiated H-2M$\alpha^{-/-}$ Recipient Mice (Thy-1.2^+)

(A) At different time points after transfer, cell suspensions from lymph nodes and/or spleen were prepared, enumerated, and triple-stained for CD4, CD8, and Thy-1.2 expression. The absolute number of recovered $CD4^+$Thy-1.2^- T cells (i.e., Thy-1.1^+) was calculated using the formula ([% $CD4^+$/Thy-1.2^- T cells] $\times$ cellularity)/100. In the experiment depicted, ammonium chloride–treated splenocytes were analyzed after adoptive transfer of 12×10^6 cells/mouse ($n = 2$ at day 8). When simultaneously analyzed, irradiated, noninjected control mice showed no detectable $CD4^+$/Thy-1.2^- T cells.

(B) Cytofluorometric analysis of cells recovered from the spleen of B6 and H-2M$\alpha^{-/-}$ recipient mice 8 days after adoptive transfer of CFSE-labeled $CD4^+$Thy-1.1^+ T cells.

(C) Purified $CD4^+$ or $CD8^+$ T cells from spleen and lymph nodes of normal B6.PL mice were adoptively transferred into irradiated B6 and H-2M$\alpha^{-/-}$ mice (10×10^6 cells/mouse). Cell suspensions were prepared from spleen and lymph nodes 8 days after transfer, enumerated, triple stained, and analyzed by cytofluorometry. The number of recovered cells was calculated as indicated above for both $CD4^+$ (left histograms) or $CD8^+$ (right histograms) T cells. The values represent the mean of two mice. For each single mouse, the calculation was the summation of recovered cells from spleen and lymph nodes.

Discussion

TCR Tg mice are convenient to analyze conditions for the maintenance of mature peripheral T cells. For example, the adoptive transfer of HY TCR Tg T cells into various recipients revealed that peripheral persistence of naive $CD8^+$ T cells requires the expression of the restricting element D^b (Tanchot et al., 1997). Mature naive $CD4^+$ T cell persistence has been analyzed in non-Tg systems. The graft of fetal thymi into RAG-2-deficient versus RAG-2/MHC II double-deficient mice revealed that the total number of newly generated $CD4^+$ T cells gradually declines over weeks in the MHC class II–deficient mice (Takeda et al., 1996). Furthermore, the transient restoration of MHC class II molecule expression in the thymic cortical epithelium of mutant mice using in situ virus-mediated gene delivery showed that positive selection and export of mature $CD4^+$ T cells to the periphery are rescued and that the long-term survival of newly generated mature $CD4^+$ T cells is impaired in the peripheral lymphoid organs in the absence of MHC class II molecules (Rooke et al., 1997). Together, these data support an emerging model in which repeated TCR-MHC interactions are required for mature peripheral T cells to persist. Such interaction results in the delivery of a survival signal remarkably reminiscent of the signal that rescues DP thymocytes from apoptosis when intrathymic positive selection occurs. Hypothesizing that the delivery of a survival signal to mature T cells is not solely based on a TCR-MHC interaction but that self-peptides may have a critical role in this process (Janeway et al., 1998), we sought to investigate the requirements for peripheral maintenance of naive mature $CD4^+$ T cells. Using a transfer-based experimental protocol, we document that naive $CD4^+$ T cells require the expression of MHC class II on APCs to persist and that this persistence is associated with a low rate of cell division. Using BALB/c recipients, we show that neither I-E$\alpha\beta^d$ or I-A$\alpha\beta^d$ heterodimers are able to ensure the persistence of I-A^b-restricted mature naive $CD4^+$ T cells. Since I-A^d is structurally close enough to I-A^b to bind Eα52-68, we conclude that, similarly to $CD8^+$ naive T cells (Tanchot et al., 1997), the expression of the restricting MHC element is required for the persistence of naive $CD4^+$ T cells.

The positive selection of MHC class II–restricted TCR Tg thymocytes on the H-2M$\alpha^{-/-}$ background (i.e., in the presence of a restricted self-peptide complexity) has been examined by several laboratories (Bevan, 1997; Grubin et al., 1997; Surh et al., 1997; Tourne et al., 1997). By including the phenotype of the 1H3.1 TCR Tg/H-2M$\alpha^{-/-}$ thymus, it appears that six of six TCRs examined to date fail to be efficiently positively selected when they confront thymic epithelial cells displaying a limited self-peptide repertoire. This is reminiscent of the impaired positive selection of $CD4^+$ thymocytes seen in the mutant mice (Martin et al., 1996) and indicates that a reduced self-peptide complexity does not permit the development of many T cells maturing normally in a wild-type thymus. It is another reflection of the critical role of self-peptides in positive selection of thymocytes evidenced by the results from single β chain TCR Tg mice (Sant'Angelo et al., 1997). The residual positive selection we observe on the H-2M$\alpha^{-/-}$ background

could theoretically be due to dual TCR thymocytes. Alternatively, it may be driven by the few non-CLIP peptides expressed by the H-2M$\alpha^{-/-}$ class II positive cells. Indeed, the direct contribution of these non-CLIP peptides to positive selection of $CD4^+$ T cells in the H-2M$\alpha^{-/-}$ mice has been demonstrated (Grubin et al., 1997).

Adoptive transfer experiments revealed an impaired maintenance of 1H3.1 TCR Tg T cells in the H-2M$\alpha^{-/-}$ recipients. This defect correlates with marginal cell division. Although it is believed that they also have an altered self-peptide complexity, we did not use the Ii$^{-/-}$ mice as recipients because their expression level of MHC class II molecules is severely impaired (Bikoff et al., 1993) and would make the interpretation difficult. This is not the case for the H-2M$\alpha^{-/-}$ mice (Martin et al., 1996; Miyazaki et al., 1996). Therefore, we conclude that the defect we observe in the H-2M$\alpha^{-/-}$ recipient mice is effectively caused by the restricted self-peptide complexity and not by a modified expression level of I-A^b molecules. The transfer of mature naive polyclonal $CD4^+$ T cells from B6 but not H-2M$\alpha^{-/-}$ donors into H-2M$\alpha^{-/-}$ recipients also reveals an impaired persistence. This further suggests that although a diverse set of Vβ domains is detected with specific mAbs (Surh et al., 1997; Tourne et al., 1997), the TCR repertoire of $CD4^+$ mature T cells is modified in the H-2M$\alpha^{-/-}$ mice. We exclude that the differences we observe reflect a $CD4^+$ T cell response to environmental antigens presented by B6 APCs and not by H-2M$\alpha^{-/-}$ APCs because it has been previously demonstrated that in the H-2M$\alpha^{-/-}$ mice, $CD4^+$ T cells are capable of responding to all antigens assayed (response to peptides, $CD4^+$ T cell response to virus epitopes and CD4-dependent production of virus-neutralizing antibody) sometimes even more efficiently than $CD4^+$ T cells from H-$2M^+$ littermates (Tourne et al., 1997).

Taken together, the 1H3.1 TCR Tg H-2M$\alpha^{-/-}$ phenotype and the transfer experiments establish that, at least for the 1H3.1 specificity, the deficient positive selection does parallel the impaired persistence on a background displaying a restricted self-peptide complexity. Thus, similar to intrathymic positive selection, the persistence of peripheral T cells is based on TCR recognition of self-peptide:self-MHC complexes. The inability of the H-2M$\alpha^{-/-}$ APCs to provide a low division signal for a significant fraction of $CD4^+$ mature T cells from H-2M$\alpha^{+/+}$ mice also supports the notion that persistence of peripheral T cells is self-peptide specific (i.e., a particular set of peptides is responsible for their persistence in the H-2M$\alpha^{+/+}$ mice). We propose that the persistence of mature T cells relies on a low level of cell division that is controlled by the TCR recognition of self-peptide:self-MHC complexes. Thus, the peripheral TCR repertoire may rely on a finely tuned balance between apoptosis and mitosis. Such a central role for self-peptides in the maintenance of mature naive T cells rules out the possibility that the survival signal delivered to naive T cells is limited to a nonspecific tickling through the CD4/CD8 coreceptor. However, this possibility may be valid for memory T cells because the expression of nonrestricting MHC class I molecules allows the persistence of transferred memory $CD8^+$ lymphocytes (Tanchot et al., 1997) and may explain the survival advantage that peripheral memory T cells have over naive T cells when confronted with recent thymic migrants (Tanchot and Rocha, 1997). Our "equilibrium model" of mature T cell persistence is in line with the finding that in thymectomized mice, T cells that retain (or regain) naive markers show a weak but detectable level of cell division (Tough and Sprent, 1994). It is known that mature thymocytes reside in the thymic microenvironment for about a week (Rooke et al., 1997) prior to emigration to the periphery. Our model also predicts that in such a delay, a low cell division of mature thymocytes should be detectable in situ. Remarkably, it has been reported that fully mature thymocytes (TCR^{high}, HSA^-, $CD4^+CD8^-$, or $CD4^-CD8^+$) produced in situ expand weakly within the thymus as demonstrated by 5-bromo-2'-deoxyuridine (BrdUrd) incorporation (Penit and Vasseur, 1997). In addition, reaggregation cultures of DP thymocytes and fetal thymic epithelial cells revealed that the fully mature SP thymocytes generated after 4–5 days undergo a significant rate of cell division (Ernst et al., 1995).

Dendritic cells (DCs) appear to be involved in delivering the survival signal to mature T cells received because the restricted expression of MHC class II molecules in DCs allows repopulation of peripheral lymphoid organs by $CD4^+$ T cells (Brocker, 1997). Additionally, a reduced number of T cells as well as a limited recovery of transferred naive $CD4^+$ T cells were observed in relB-deficient mice (DeKoning et al., 1997) that lack DCs (Burkly et al., 1995). Such a role for DCs is consistent with the fact that they regularly interact with recirculating T cells in the T cell areas. Interestingly, besides the peripheral DCs that migrate to the T cell areas to induce immune responses, recent observations suggest that another set of DCs seem to reside in situ. This subset, which express self-peptide:MHC complexes, could be involved in the maintenance of tolerance (Steinman et al., 1997). We speculate that it may also be crucially involved in the long-term persistence of mature T cells. The contribution of B cells to $CD4^+$ T cell maintenance is unknown. Such contribution may be limited since it was demonstrated that mature T cells transferred into SCID mice survive normally (Sprent et al., 1991).

Thus, our experiments demonstrate that both the correct MHC, in this case I-A^b, and the correct self-peptide, in this case shown by the effects of the absence of the MHC class II peptide exchange factor H-2M, are required for maintenance of $CD4^+$ T cells in the periphery. Also shown is that the $CD4^+$ T cells persist over time due to a low rate of cell division, which is presumably balanced by apoptosis although this was not observed directly. Finally, we found that the polyclonal $CD4^+$ T cells positively selected in H-2M$\alpha^{-/-}$ mice can persist in H-2M$\alpha^{-/-}$ recipients but fail to do so in the absence of MHC class II. These data are consistent with a role for self-peptides:self-MHC class II complexes not only in the thymus but also in the periphery for maintaining the naive TCR repertoire.

Experimental Procedures

Animals

Mice used were housed in the Yale immunobiology facility. C57BL/6 (B6) mice and congenic mice expressing the CD90.1/Thy1.1 allele (B6. PL-Thy1a/Cy) were obtained from the Jackson Laboratory. The

thymectomized B6, B6 I-A$^b\beta^{-/-}$ (MHC class II–deficient [Grusby et al., 1991]), B6 $\beta2m^{-/-}$ (MHC class I–deficient [Zijlstra et al., 1990]), and BALB/c mice were purchased from Taconic. The 1H3.1 TCR Tg mice (Vα1-Jα21/Vβ6-Dβ2.1-Jβ2.6) were generated in this laboratory (C. V. and C. A. J., unpublished data) and are maintained on a B6 background. H-2M$\alpha^{-/-}$ mice (B6-129 mixed background) and oligonucleotide sequences for PCR-based genotyping were provided by Dr. L. Van Kaer (HHMI). The I-A^b-Ep mice (Ignatowicz et al., 1996) were a gift of Dr. P. Marrack (HHMI). The RAG-1-deficient mice were a gift of Dr. D. Shatz (HHMI, Yale University).

Adoptive Transfer

1H3.1 TCR Tg CD4$^+$ T cells, polyclonal H-2M$\alpha^{-/-}$ CD4$^+$ and B6 CD4$^+$ Thy1.1$^+$ T cells from lymph nodes and spleen were prepared as single cell suspensions and purified using magnetic beads (Bio Mag, Advanced Magnetic) and the Y3JP (anti-I-A^b), TIB 164 (anti-B220), TIB 105, and TIB 210 (anti-CD8) mAbs to remove APCs and CD8$^+$ T cells without delivering signal to T cells through the TCR or coreceptor. The efficiency of this purification was assessed by CD4/Vβ6 or CD4/$\alpha\beta$TCR double staining, and purity ranged from 92% to 96%. Cells were washed three times and resuspended in normal saline. Injections were done intravenously into the retroorbital plexus of the eye using 5–8 $\times$ 10^6 cells/200 μl per mouse unless otherwise indicated. Six- to ten-week-old recipient animals used were thymectomized and sublethally irradiated (600 rads, ^{137}Cs source, Yale University Cancer Center) unless otherwise indicated. In some experiments, purified mature T cells were dye labeled prior to transfer using 5, 6-carboxy-succinimidyl-fluorescein-ester (CFSE [Molecular Probes]). Labeling was performed in normal saline at 10^7 cells/ml using 2 μl of a 5 mM CFSE stock solution for 10 min at 37°C. Cells were washed twice in saline and injected as indicated above. The labeling efficiency was checked by FACS (fluorescence in FL1) prior to injection. In all transfer experiments, the donor and recipient were sex matched.

Immunostaining and Flow Cytometry

Depending on experiments, thymus, spleen, and lymph nodes (axillary, lateral axillary, superficial inguinal, and mesenteric) were removed and cell suspensions prepared. Splenic red blood cells were lysed using Tris-buffered ammonium chloride. Fluorescent labeled mAbs were used for multicolor staining. In brief, 0.2 $\times$ 10^6 cells were incubated in microtiter U-bottom plates with saturating concentrations of labeled mAb in 20 μl for 30 min on ice. Cells were washed twice and analyzed immediately without fixation. For two-step staining, cells were incubated first with purified mAbs in PBS 2% FCS/0.1% NaN3, followed by a F(ab')$_2$ fragment of goat anti-mouse Ig-Fluorescein isothiocyanate (FITC) conjugate from Sigma Chemical. The mAbs used were anti-Vβ6-FITC (clone RR4-7), anti-Cβ-Phycoerythrin (PE) (H57-597), anti-Vα2,3.2,8,11-FITC (B20.1, RR3-16, B21.14, RR8-1), anti-CD90.2/Thy-1.2-PE (clone 53-2.1 not cross-reactive to Thy-1.1) from Pharmingen, anti-CD8α-PE/FITC (53-6.7) from GIBCO-BRL, and anti-CD4-quantum red (H129.19) from Sigma. The Y3JP (mouse IgG$_{2a}$, anti-I-A^b) (Janeway et al., 1984), 25.9.17 (mouse IgG$_{2a}$, anti-I-A^b) (Ozato et al., 1980), Y-A*e* (mouse IgG$_{2b}$, anti-A^b+Eα) (Murphy et al., 1989), GK1.5 (rat IgG$_{2b}$, anti CD4), TIB 105, and TIB 210 (both rat IgG$_{2b}$, anti CD8) mAbs were affinity purified in the laboratory using standard procedures. A FACSCAN flow cytometer and the CellQuest software from Becton Dickinson were used to collect and analyze the data. Nonviable cells were excluded using forward and side scatter electronic gating.

Functional Assays

For T cell proliferation assay, T cell suspensions were prepared from lymph nodes and cultured in U-bottom 96-well plates for (Becton-Dickinson) 3–4 days at 37°C in Click's EHAA medium (Irvine Scientific) supplemented with 5% heat-inactivated fetal calf serum (Intergen), 5 $\times$ 10^{-5} M 2-mercaptoethanol (Biorad), 2 mM L-glutamine, and 50 μg/ml gentamicin (GIBCO). Depending on the experiment, T cells (30–50 $\times$ 10^3/well) were stimulated using irradiated B6 splenocytes as APCs (3 $\times$ 10^5 or less/well, 2000 rad) plus serial dilutions of synthetic Eα52-68 peptide (ASFEAQGALANIAVDKA; single letter amino acid code) in a total volume of 150 μl. The cells were incubated in duplicate wells and 1 μCi of ^{3}H thymidine/well was added to the culture during the last 12 hr. The plates were then harvested and counts/minute were determined using liquid scintillation counting. For inhibition experiments, purified monoclonal antibodies (3–5 μg/ml) were sterile filtered and added to microcultures.

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References

Ashton-Rickardt, P.G., Van Kaer, L., Schumacher, T.N.M., Ploegh, H.L., and Tonegawa, S. (1993). Peptide contributes to the specificity of positive selection of CD8+ T cells in the thymus. Cell *73*, 1041–1049.

Bevan, M.J. (1997). In thymic selection, peptide diversity gives and takes away. Immunity *7*, 175–178.

Bikoff, E.K., Huang., L.Y., Episkopou, V., van Meerwijk., J., Germain, R.N., and Robertson, E.J. (1993). Defective MHC complex class II assembly, transport, peptide acquisition and CD4+ T cell selection in mice lacking invariant chain expression. J. Exp. Med. *177*, 1699–1712.

Brocker, T. (1997). Survival of mature CD4 T lymphocytes is dependent on MHC class II-expressing dendritic cells. J. Exp. Med. *186*, 1223–1232.

Bruno, L., Kirberg, J., and von Boehmer, H. (1995). On the cellular basis of immunological T cell memory. Immunity *2*, 37–43.

Burkly, L., Hession, C., Ogata, L., Reilly, C., Marconi, L.A., Olson, D., Tizard, R., Cate, R., and Lo, D. (1995). Expression of relB is required for the development of thymic medulla and dendritic cells. Nature *373*, 531–536.

Chervonsky, A.V., Medzhitov, R.M., Denzin, L.K. , Barlow, A.K., Rudensky, A.Y., and Janeway, C.A., Jr. (1998). Subtle conformational changes induced in major histocompatibility complex class II molecules by binding peptides. Proc. Natl. Acad. Sci. USA. *95*, 10094–10099.

DeKoning, J., DiMolfetto, L., Reilly, C., Wei, Q., Havran, W.L., and Lo, D. (1997). Thymic cortical epithelium is sufficient for the development of mature T cells in relB-deficient mice. J. Immunol. *158*, 2558–2566.

Denzin, L.K., and Cresswell, P. (1995). HLA-DM induces CLIP dissociation from MHC class II $\alpha\beta$ dimers and facilitates peptide loading. Cell *82*, 155–165.

Ernst, B., Surh, C.D., and Sprent, J. (1995). Thymic selection and cell division. J. Exp. Med. *182*, 961–972.

Fung-Leung, W.P., Surh, C.D., Liljedahl, M., Pang, J., Leturcq, D., Peterson, P.A., Webb, S.R., and Karlsson, L. (1996). Antigen presentation and T cell development in H2-M deficient mice. Science *271*, 1278–1281.

Grubin, C.E., Kovats, S., deRoos, P., and Rudensky, A.Y. (1997). Deficient positive selection of CD4$^+$ T cells in mice displaying altered repertoire of MHC class II bound self-peptides. Immunity *7*, 197–208.

Grusby, M., Johnson, R.S., Papaioannou, V.E., and Glimcher, L.H. (1991). Depletion of CD4+ T cells in major histocompatibility complex class II deficient mice. Science *253*, 1417–1420.

Hogquist, K.A., Gavin, M.A., and Bevan, M.J. (1993). Positive selection of CD8+ T cells induced by major histocompatibility complex binding peptides in fetal thymic organ culture. J. Exp. Med. *177*, 1469–1473.

Hunt, D.F., Michel, H., Dickinson, T.A., Shabanowitz, J., Cox, A.L., Sakaguchi, K., Apella, E., Greg, H.M., and Sette, A. (1992). Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-Ad. Science *256*, 1817–1820.

Ignatowicz, L., Kappler, J., and Marrack, P. (1996). The repertoire of T cells shaped by a single MHC/peptide ligand. Cell *84*, 521–529.

Jameson, S.C., Hogquist, K.A., and Bevan, M.J. (1995). Positive selection of thymocytes. Annu. Rev. Immunol. *13*, 93–126.

Janeway, C.A., Jr., Conrad, P.J., Lerner, E.A., Babich, J., Wettstein, P., and Murphy, D.B. (1984). Monoclonal antibodies specific for Ia glycoproteins raised by immunization with activated T cells: possible role of T cell bound Ia antigens as targets of immunoregulatory T cells. J. Immunol. *132*, 662–667.

Janeway, C.A., Jr., Kupfer, A., Viret, C., Boursalian, T., Goverman, J., Bottomly, K., and Sant'Angelo, D.B. (1998). T cell development, survival and signaling: a new concept of the role of self peptide:self MHC complexes. Immunologist *6*, 5–12.

Kirberg, J., Berns, A., and von Boehmer, H. (1997). Peripheral T cell survival requires continual ligation of the T cell receptor to MHC-encoded molecules. J. Exp. Med. *186*, 1269–1275.

Kisielow, P., and von Boehmer, H. (1995). Development and selection of T cells: facts and puzzles. Adv. Immunol. *58*, 87–209.

Lam, K.P., Kuhn, R., and Rajewsky, K. (1997). In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell *90*, 1073–1083.

Lyons, A.B., and Parish, C.R. (1994). Determination of lymphocyte division by flow cytometry. J. Immunol. Methods *171*, 131–137.

Martin, W.D., Hicks, G.G., Mendiratta, S.K., Leva, H.I., Ruley, E.H., and Van Kaer, L. (1996). H-2M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. Cell *84*, 543–550.

Miyazaki, T., Wolf, P., Tourne, S., Waltzinger, C., Dierich, A., Barois, N., Ploegh, H., Benoist, C., and Mathis, D. (1996). Mice lacking H2-M complexes, enigmatic elements of the MHC class Ii peptide loading pathway. Cell *84*, 531–541.

Murphy, D.B., Lo, D., Rath, S., Brinster, R.L., Flavell, R.A., Slanetz, A., and Janeway, C.A., Jr. (1989). A novel MHC class II epitope expressed in thymic medulla but not cortex. Nature *338*, 765–768.

Murphy, D.B., Rath, S., Pizzo, E., Rudensky, A.Y., George, A., Larson, J.K., and Janeway, C.A., Jr. (1992). Monoclonal antibody detection of a major self peptide. MHC class II complex. J. Immunol. *148*, 3483–3491.

Neuberger, M.S. (1997). Antigen receptor signaling gives lymphocytes a long life. Cell *90*, 971–973.

Nikolic-Zugic, J., and Bevan, M.J. (1990). Role of self-peptides in positively selecting the T cell receptor repertoire. Nature *344*, 65–67.

Nossal, G.J.V. (1994). Negative selection of lymphocytes. Cell *76*, 229–240.

Ozato, K., Mayer, N., and Sachs, D.H. (1980). Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J. Immunol. *124*, 533–540.

Penit, C., and Vasseur, F. (1997). Expansion of mature thymocytes subsets before emigration to the periphery. J. Immunol. *159*, 4848–4856.

Riberdy, J.M., Newcomb, J.R., Surman, M.J., Barbosa, J.A., and Creswell, P. (1992). HLA-DR molecules from an antigen-processing mutant cell line are associated with invariant chain peptides. Nature *353*, 660–662.

Robey, E., and Fowlkes, B.J. (1994). Selective events in T cell development. Annu. Rev. Immunol. *12*, 675–705.

Rocha, B., and von Boehmer, H. (1991). Peripheral selection of the T cell repertoire. Science *251*, 1225–1228.

Rooke, R., Waltzinger, C., Benoist, C., and Mathis, D. (1997). Targeted complementation of MHC class II deficiency by intrathymic delivery of recombinant adenovirus. Immunity *7*, 123–134.

Rudensky, A.Y., Rath, S., Preston-Hurlburt, P., Murphy, D.B., and Janeway, C.A., Jr. (1991a). On the complexity of self. Nature *353*, 660–662.

Rudensky, A.Y., Preston-Hurlburt, P., Hong, S.C., Barlow, A., and Janeway, C.A., Jr. (1991b). Sequence analysis of peptides bound to MHC class II molecules. Nature *353*, 622–627.

Sant'Angelo, D.B., Waterbury, P.G., Cohen, B.E., Martin, W.D., Van Kaer, L., Hayday, A.C., and Janeway, C.A., Jr. (1997). The imprint of intrathymic self-peptides on the mature T cell receptor repertoire. Immunity *7*, 517–524.

Sette, A., Ceman, S., Kubo, R.T., Sakaguchi, E., Apella, K., Hunt, D.F., Davis, T.A., Michrel, H., Shabanowitz, J., Rudersdorf, R., et al. (1992). Invariant chain peptides in most HLA-DR molecules of an antigen-processing mutant. Science *258*, 1801–1804.

Sha, W.C., Nelson, C.A., Newberry, R.D., Pullen, J.K., Pease, L.R., Russell, J.H., and Loh, D.Y. (1990). Positive selection of transgenic receptor-bearing thymocytes by Kb antigen is altered by Kb mutations that involve peptide binding. Proc. Natl. Acad. Sci. USA *87*, 6186–6190.

Sprent, J., Schaefer, M., Hurd, M., Surh, C.D., and Ron, Y. (1991). Mature murine B and T cells transferred to SCID mice can survive indefinitely and many maintain a virgin phenotype. J. Exp. Med. *174*, 717–728.

Steinman, R.M., Pack, M., and Inaba, K. (1997). Dendritic cells in the T cell areas of lymphoid organs. Immunol. Rev. *156*, 25–37.

Surh, C.D., Lee, D.S., Fung-Leung, W.P., Karlsson, L., and Sprent, J. (1997). Thymic selection by a single MHC/peptide ligand produces a semidiverse repertoire of CD4+ T cells. Immunity *7*, 209–219.

Takeda, S., Rodewald, H.R., Arakawa, H., Bluethmann, H., and Shimizu, T. (1996). MHC class II molecules are not required for survival of newly generated CD4+ T cells but affect their long-term life span. Immunity *5*, 217–228.

Tanchot, C., and Rocha, B. (1995). The peripheral T cell repertoire: independent homeostatic regulation of virgin and activated CD8+ T cell pools. Eur. J. Immunol. *25*, 2127–2136.

Tanchot, C., and Rocha, B. (1997). Peripheral selection of T cell repertoires: the role of continuous thymus output. J. Exp. Med. *7*, 1099–1106.

Tanchot, C., Lemonier, F.A., Perarnau, B., Freitas, A.A., and Rocha, B. (1997). Differential requirements for survival and proliferation of CD8 naive or memory T cells. Science *276*, 2057–2062.

Tough, D.F., and Sprent, J. (1994). Turnover of naive- and memory-phenotype T cells. J. Exp. Med. *179*, 1127–1135.

Tourne, S., Miyazaki, T., Oxenius, A., Klein. L., Fehr, T., Kyewski, B., Benoist, C., and Mathis, D. (1997). Selection of a broad repertoire of CD4+ T cells in mice H-2M$\alpha^{o/o}$ mice. Immunity *7*, 187–196.

Viret, C., and Janeway, C.A., Jr. (1999). MHC and T cell development. Rev. Immunogenet. *1*, in press.

von Boehmer, H. (1994). Positive selection of lymphocytes. Cell *76*, 219–228.

von Boehmer, H., and Hafen, K. (1993). The lifespan of naive a/b T cells in secondary lymphoid organs. J. Exp. Med. *177*, 891–896.

Webb, S.R., Morris, C., and Sprent, J. (1990). Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. Cell *63*, 1249–1256.

Zijlstra, M., Bix, M., Simister, N.E., Loring, J.M., Raulet, D.H., and Jaenisch, R. (1990). β2-microglobulin deficient mice lack CD4-CD8+ cytolytic T cells. Nature *344*, 742–746.