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Mature CD4$^+$ T Cells Perceive a Positively Selecting Class II MHC/Peptide Complex in the Periphery[1]

Pawel Muranski, Bartosz Chmielowski, and Leszek Ignatowicz[2]

A repertoire of TCRs is selected in the thymus by interactions with MHC bound to self-derived peptides. Whether self peptides bound to MHC influence the survival of mature T cells in the periphery remains enigmatic. In this study, we show that the number of naive CD4$^+$ T cells that developed in mice with class II MHC bound with endogenous peptides (A^{b}wt) diminished when transferred into mice with A^b covalently bound with a single peptide (A^{b}Ep). Moreover, transfer of a mixture of naive CD4$^+$ T cells derived from A^{b}wt and from A^{b}Ep mice into A^{b}Ep mice resulted in the expansion of the latter and decline of the former. In contrast, when wild-type activated CD4$^+$ T cells were transferred into A^{b}Ep or A^{b}wt mice, these cells survived in both recipients for more than 4 wk, but further expanded in the A^{b}wt host. We conclude that to survive, naive CD4$^+$ T cells favor peripheral expression of the class II MHC/peptide complex(es) involved in their thymic selection, whereas some of activated CD4$^+$ T cells may require them only for expansion. *The Journal of Immunology*, **2000, 164: 3087–3094.**

In the thymus, immature T cells are positively and negatively selected via their Ag receptors (TCRs) if engaged by self MHC/peptide complexes expressed on thymic stromal cells (1, 2). Positive selection biases the repertoire of TCRs on thymocytes toward ones that can bind self MHC/peptide ligands with low affinity (3), while negative selection eliminates thymocytes with TCRs that bind self MHC/peptide ligands with high affinity (4). Thymocytes with TCRs unable to perceive self MHC/peptide complexes die by neglect because they are inherently useless. Mature thymocytes that successfully pass both selections leave the thymus, repopulate the periphery, and can remain quiescent for weeks, unless challenged with a specific Ag that leads to their further differentiation into effector and/or memory cells (5–7). Unsolved issue remains as to whether sustained survival of naive T cells in the periphery also depends on TCR-mediated signals. By analogy to thymic positive selection, it was postulated that interactions that extend the survival of naive T cells in the periphery are mediated via TCRs that continuously recognize self MHC/peptide complexes with a low affinity that is adequate to maintain, but not to activate, naive T cells (8). There is considerable experimental evidence showing that the prolonged survival of naive CD8$^+$ and CD4$^+$ T cells is contingent upon the expression of class I and class II MHC molecules, respectively (8–12). Moreover, transferred naive T cells survived longer in sublethally irradiated recipients expressing MHC alleles identical to ones that originally selected these T cells in thymus (8, 13). Thus, it appears that, at least for naive T cells, continuous contact with self MHC in the periphery is prerequisite for their survival. However, whether these low affinity interactions involve the recognition of self MHC or also a particular peptide bound to MHC remains to be determined. Recently, mice expressing class II MHC molecules occupied either with a dominant peptide or a single peptide were found to be able to support the development of 15–40% of the wild-type number of CD4$^+$ T cells (14–17). Moreover, CD4$^+$ T cells survive unhindered for the life span of these mice, indicating that A^b bound with one peptide is competent to condition the prolonged survival of these CD4$^+$ T cells (Ref. 16; P. Muranski, unpublished data). To determine whether mature CD4$^+$ T cells perceive the diversity of self-derived peptides bound to MHC, we followed survival of wild-type CD4$^+$ T cells adoptively transferred into a recipient with no class II MHC, with class II MHC bound with one peptide, or with wild-type class II MHC bound with many peptides. Our results show that for prolonged survival, naive CD4$^+$ T cells require both self MHC and peptides that initially were involved in their thymic selection. Only a few of the transferred naive CD4$^+$ T cells from wild-type mice survived for 4 wk in the periphery of sublethally irradiated, single class II MHC/peptide mice or class II MHC-deficient mice, while the same CD4$^+$ T cells rapidly expanded in a wild-type recipient. In contrast, in vitro activated CD4$^+$ T cells derived from wild-type mice persisted unchanged in the periphery of single class II MHC/peptide mice for 4 wk, but expanded better in the presence of wild-type peptides bound to A^b. Therefore, the presence of a diverse range of self peptides bound to MHC molecules is important not only for the efficient selection of thymocytes, but also for the extended survival of mature T cells.

Materials and Methods

Animals

Mice expressing single A^{b}Ep complex with invariant chain (A^{b}EpIi$^+$)[3] or without invariant chain (A^{b}EpIi$^-$) and deficient in the expression of wild-type A^b molecules were generated at the National Jewish Medical and Research Center (Denver, CO). These animals and MHC class II-deficient (A^{b-}), A^{b}EpIi$^-\beta_2$m$^-$, and F$_1$ (A^{b}EpIi$^-$ × A^{b}wt) mice were further bred in the animal facility at the Medical College of Georgia (Augusta, GA). Animals deficient in MHC class I and II (MHC$^-$) and animals deficient in CD4 molecules were also bred in the above facility.

Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA 30912

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[2] Address correspondence and reprint requests to Dr. Leszek Ignatowicz, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA 30912-2600. E-mail address: ignatowi@immagene.mcg.edu

[3] Abbreviations used in this paper: Ii, invariant chain; β_2m, β_2-microglobulin; CFSE, succinimidyl ester of carboxyfluorescein diacetate.

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C57BL/6 and congenic mice B6.PL-Thy-1a/Ca expressing the CD90.1/Thy-1.1 Ag were purchased from The Jackson Laboratory (Bar Harbor, ME).

Radiation bone marrow chimeras

Bone marrow from donor mice was depleted of mature T cells using anti-Thy-1.2 mAb (HO 13.4.6) and complement and was subsequently checked for purity by FACS. Recipient mice were lethally irradiated (1100 R) and reconstituted within 6 h with 5×10^6 cells. If not otherwise stated, radiation chimeras were used for experiments at least 3 wk postreconstitution.

Complement depletion

Single cell suspensions from pooled axillary, inguinal, mesenteric, and paraaortic lymph nodes were prepared and incubated for 30 min at 4°C with cytotoxic mixture prepared from supernatants or with purified Abs from the following hybridomas cultured in this laboratory: anti-CD8 (clone HO 2.2), anti-MHC class II (clones 25-6-3S and BP107.2.2), anti-CD45 (clone B220), and antiJ11D (clone J11D.2). Cells were then incubated at 37°C with a mixture of rabbit (Sigma, St. Louis, MO) and guinea pig (Life Technologies, Grand Island, NY) complement. After a single washing, cells were checked by flow cytometry for the presence of cells bearing CD8 or class II MHC (A^b). Purity of more than 90% CD4$^+$ cells was achieved with no A^b cells present.

In vitro stimulation

Plastic flasks were coated overnight with anti-TCR Ab (HAM 57.597.2) in balanced salt solution and washed once. Purified CD4$^+$ T cells were cultured in them for 6 days with addition of IL-2 on day 3.

Cell tracking

For tracking lymphocytes in vivo, a lipophilic succinimidyl ester of carboxyfluorescein diacetate (CFSE) purchased from Molecular Probes (Eugene, OR) was used. CFSE passively enters the cytoplasm, where it is rapidly hydrolyzed into a fluorescent hydrophilic metabolite that is unable to diffuse out and is readily detectable by FACS. CFSE concentration decreases with each cell division.

Adoptive transfers

CD4$^+$ lymphocytes, purified by complement depletion, were suspended at 5×10^6/ml in PBS, and CFSE was added to a final concentration of 1 μg/ml and incubated at 37°C for 15 min. Cells were washed, suspended in PBS, counted, and injected i.v. into recipient animals, which, if not otherwise stated, had been sublethally (600 rad) irradiated.

Flow cytometry

The biotin-, fluorescein-, or PE-labeled mAbs were prepared in this laboratory or purchased from PharMingen (San Diego, CA). Cells were suspended in blocking solution (50% culture supernatant of anti-FcR Ab 2.4G2 and 50% FBS) and incubated (4°C, 30 min) with Abs of interest washed twice and analyzed using a FACSCalibur flow cytometer (Becton Dickinson, San Diego, CA).

Quantitation of total cell number

Single cell suspensions were prepared from the pooled axillary, inguinal, mesenteric, and paraaortic lymph nodes and spleen (treated with ammonium chloride buffer to lyse erythrocytes), and cell numbers were measured. Percentage of CD4$^+$ T lymphocytes of interest (depending on experiment: CFSE$^+$, CFSE$^-$, Thy-1.1$^+$, Thy-1.1$^-$) in each animal was evaluated using flow-cytometric analysis. Total number was obtained by adding the number of cells in spleen and twice the number found in lymph nodes, as described (18, 19). For the purpose of these calculations, all cells outside of the clearly negative group were considered to be CFSE$^+$. There were lymphocytes with high and low levels of fluorescence among them, but no such distinction was made.

Results

Wild-type CD4$^+$ T cells survive unimpaired if transferred in sublethally irradiated recipients expressing high or low levels of A^b bound with many self-derived peptides

The present study was undertaken to determine whether the diversity of self-derived peptides bound to class II MHC influences the persistence of CD4$^+$ T cells in the periphery as it does in the thymus. It is known that naive CD4$^+$ T cells continuously sense the presence of self MHC molecules in the periphery, but whether particular self peptides bound to MHC are also specifically recognized during these interactions has not been investigated. To approach this question, we intended to use, as recipients for adoptively transferred wild-type CD4$^+$ T cells, mice that lack endogenous A$^b\beta$-chain together with an Ii chain and that are transgenic for A$^b\beta$ covalently attached with single peptide Eα (52–63) (A^{b}EpIi$^-$ mice). A potential caveat for using A^{b}EpIi$^-$ mice in these experiments is a lower expression of the transgenic single A^{b}Ep complex in comparison with the A^b bound with endogenous peptides in wild-type mice (20). Hence, one may be concerned that the expression level of A^{b}Ep complex may be insufficient for CD4$^+$ T cells that developed in mice expressing a normal level of A^b. Therefore, to test whether lower expression of the transgenic A^b will support the survival of wild-type CD4$^+$ T cells if bound with endogenous self peptides, we transferred these T cells into transgenic A^{b}EpIi$^+$ mice. In the A^{b}EpIi$^+$ mice, the covalent A^{b}Ep complex is unstable due to an Ii chain that directs the A^{b}Ep to endosomes, where covalent peptide is cleaved and replaced with the diverse set of self-derived peptides (21). Because of that, A^{b}EpIi$^+$ mice that express the same lower levels of transgenic A^b as A^{b}EpIi$^-$ mice select and maintain a high number of CD4$^+$ T cells in the thymus and in the periphery (20). As shown in Fig. 1, wild-type CD4$^+$ T cells labeled with fluorescent dye (CFSE) and adoptively transferred to sublethally irradiated A^{b}EpIi$^+$ mice or to wild-type mice proliferated with similar kinetics (10 and 30 days after transfer), indicating that transferred CD4$^+$ T cells perceive both low and high numbers of A^b if bound with many peptides. This result indicated that transgenic A^b bound to self peptides is sufficient to support the survival of adoptively transferred CD4$^+$ T cells from mice expressing A^{b}wt.

Survival of the naive CD4$^+$ T cells derived from A^{b}wt mice depends on contact with A^b bound to self peptides

Next, we sought to determine whether CD4$^+$ T cells that matured in wild-type mice would survive in the presence of a single A^{b}Ep complex. Three different groups of sublethally irradiated recipients, differing in the complexity of self peptides bound to A^b, were used. The first group of mice lacked expression of CD4 and had no CD4$^+$ T cells in the periphery, but expressed intact class II MHC molecules (A^b) loaded with a diverse set of wild-type peptides (22). The second group of mice was A^{b}EpIi$^-$ mice with all A^b molecules covalently bound with the single peptide (16). The third type of recipient mice had no expression of A^b molecules and very few peripheral CD4$^+$ T cells (23). All these groups of mice were injected with CFSE-labeled wild-type CD4$^+$ T cells bearing a different allele of Thy-1 Ag to follow their fate regardless of CFSE label. As shown on the *left panel* in Fig. 2*A*, after 2 days, all detectable CD4$^+$ Thy-1.1$^+$ T cells were positive for CFSE in all recipients. Four weeks later, one-third of CD4$^+$ Thy-1.1$^+$ T cells transferred to A^{b}wt mice were CFSE positive, while 75% and 88% remained CFSE positive in the A^{b}EpIi$^-$ or A^{b-} recipients, respectively. After 30 days, recipients were sacrificed and the total numbers of recovered CD4$^+$ Thy1.1$^+$ T cells were calculated. We found that transferred CD4$^+$ T cells in wild-type mice significantly expanded, whereas in the two other types of recipients, the same transferred cells decayed by more than 70% (Fig. 2*B*). The number of CSFE$^+$ T cells was also highest in the A^{b}wt recipients because this population included many continuously expanding cells that had not yet completely lost the CSFE staining (Fig. 2*B*). As shown in Fig. 3*A*, 90% of CD4$^+$ T cells isolated from B6.PL mice had a naive phenotype (CD44^{low}CD45^{high}), but when transferred into

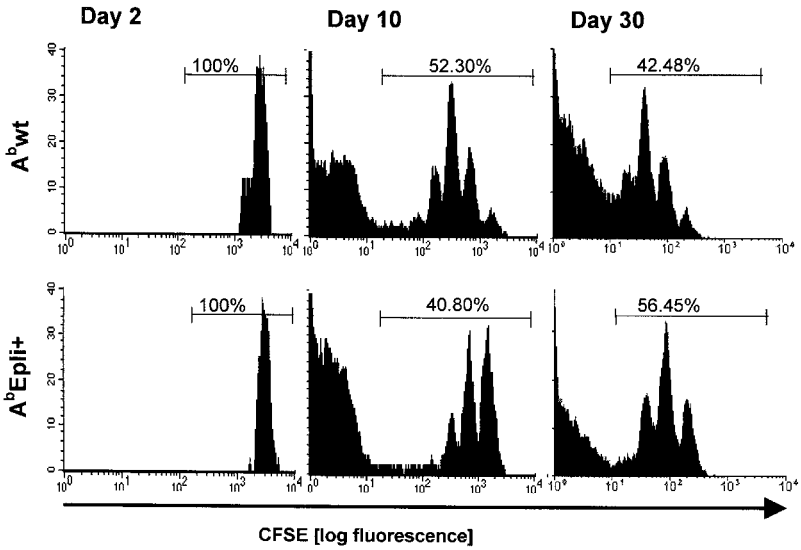

FIGURE 1. Expansion of adoptively transferred CD4$^+$ cells from A^{b}wt donors exposed to low level of MHC class II occupied with a heterogeneous repertoire of peptides. A total of 3×10^6 purified CD4$^+$ lymphocytes from B6.PL (Thy-1.1$^+$) donors were labeled with CFSE and transferred into sublethally irradiated A^{b}EpIi$^+$ and C57BL/6 (A^{b}wt) recipients. Examples of FACS analysis of peripheral blood on days 2, 10, and 30 are shown. Histograms are plotted after gating on Thy-1.1$^+$ CD4$^+$ cells.

syngenic wild-type recipients, the expanding cells up-regulated CD44 and down-regulated CD45RB expression (Fig. 3, *B* and *C*). In contrast, in two other types of recipients that expressed a single class II MHC/peptide complex or had no class II MHC, the majority of transferred CD4$^+$ Thy-1.1$^+$ cells decayed after 30 days with at most an unchanged phenotype. Expression of activation markers after adoptive transfer of naive TCR transgenic, CD4$^+$ T cells into a syngenic irradiated recipient has been previously reported (24, 25). These cells sustained elevated expression of CD44 in the absence of Ag for few weeks. Our experiments confirm this phenomenon, and additionally show that to survive and to expand, naive wild-type CD4$^+$ T cells require a diverse set of self-derived peptides bound to class II MHC molecules.

To survive, peripheral, naive CD4$^+$ T cells favor contact with the self A^b/peptide complex encountered during thymic selection

The fact that the majority of wild-type derived CD4$^+$ T cells quickly disappeared when transferred into the in vivo environment of class II MHC preloaded with one peptide contrasted with the extended survival of naive CD4$^+$ T cells present in the single class II MHC/peptide mice (Ref. 16; P. Muranski, unpublished data). This observation could indicate that one of the self A^b/peptide complexes that may provide the survival signal to naive CD4$^+$ T cells is the same self-derived peptide(s) that bound to the class II MHC that positively selected them in the thymus. To test this hypothesis, we have performed two adoptive transfer experiments.

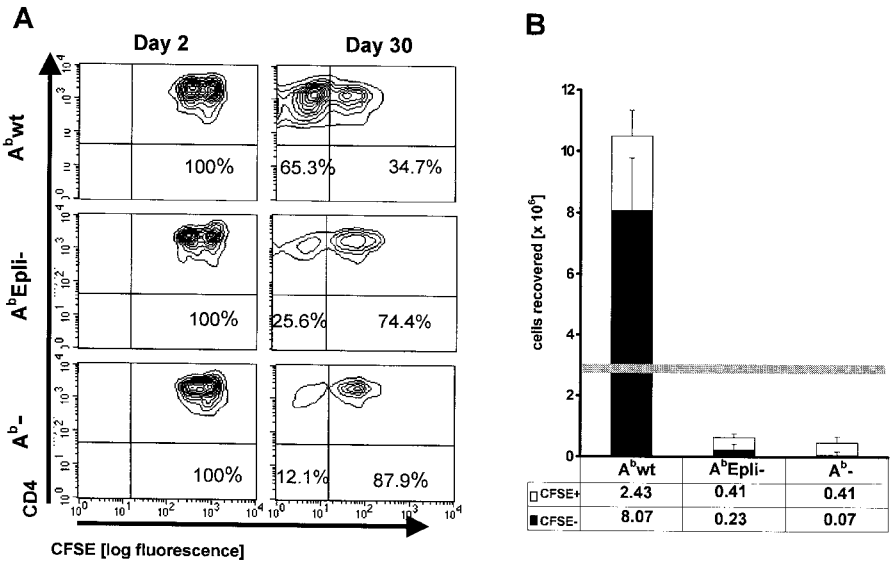

FIGURE 2. Naive CD4$^+$ lymphocytes selected on wild-type MHC class II require exposure to A^{b}wt in the periphery to survive and expand. A total of 3×10^6 B6.PL (Thy-1.1$^+$) CD4$^+$ T cells were purified, labeled with CFSE, and adoptively transferred into sublethally irradiated recipients with no, or low numbers of, intrinsic Thy-1.2$^+$ CD4$^+$ lymphocytes: A^{b}wt (CD4$^-$), A^{b}EpIi$^-$, A^{b-}. *A*, Example of FACS analysis representative for 10 experiments performed. Peripheral blood was treated with ammonium chloride buffer and stained with mAbs anti-CD4 biotin and anti-Thy-1.1 PE. As a third color (FL1), CFSE was used. Contour plots illustrate proliferation of transferred lymphocytes (gated on Thy-1.1$^+$ CD4$^+$ cells) on days 2 and 30. *B*, Total numbers of Thy-1.1$^+$ CD4$^+$ T lymphocytes recovered after 30 days posttransfer from peripheral lymphatic organs with the proportion of cells retaining (CFSE$^+$) and lacking (CFSE$^-$) fluorescent dye. Table provides numeric value ($\times 10^6$). Grey line shows number of cells transferred (3×10^6). A total of 6–10 animals per each experimental group were used.

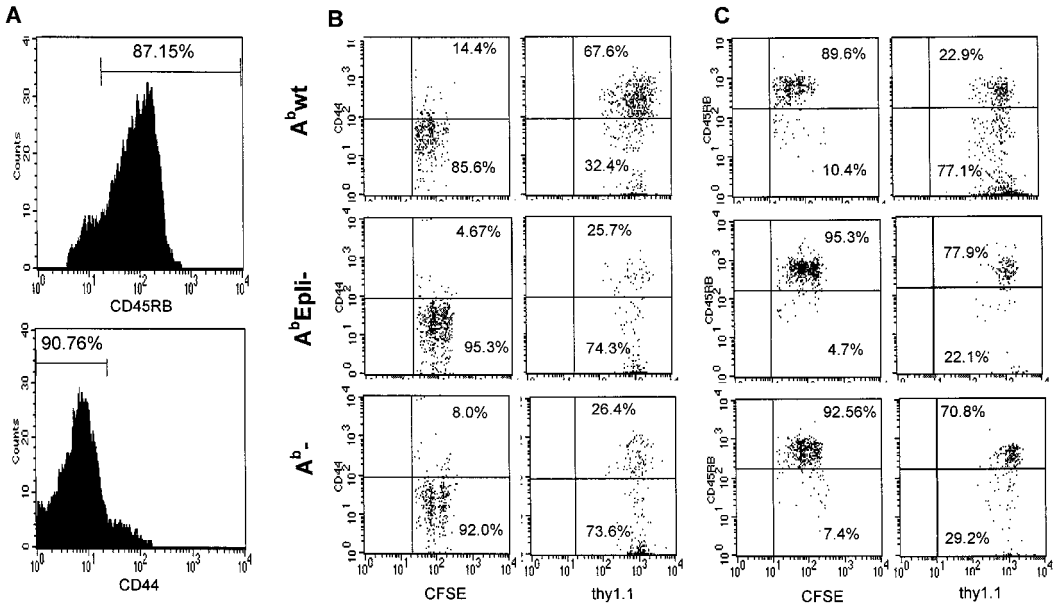

FIGURE 3. Expression of surface molecules CD44 and CD45RB on Thy-1.1$^+$ CD4$^+$ cells recovered after transfer into animals with various MHC class II environments. A total of 3×10^6 B6.PL (Thy-1.1$^+$) CD4$^+$ T cells were purified, labeled with CFSE, and adoptively transferred into sublethally irradiated recipients with no, or low numbers of, intrinsic Thy-1.2$^+$ CD4$^+$ lymphocytes: A^{b}wt (CD4$^-$), A^{b}EpIi$^-$, A^{b-}. *A*, Expression of CD44 and CD45 on CD4$^+$ T cells before adoptive transfer. *B*, Expression of CD44 on Thy-1.1$^+$ CD4$^+$ cells recovered from lymph nodes after 30 days postadoptive transfer into sublethally irradiated A^{b}wt, A^{b}EpIi$^-$, and A^{b-} recipients. *Left column* shows expression of CD44 on slowly dividing cells that retained fluorescent dye (CFSE$^+$ CD4$^+$). *Right column* depicts expression of CD44 on all recovered Thy-1.1$^+$ CD4$^+$ cells. *C*, Expression of CD45RB on Thy-1.1$^+$ CD4$^+$ T cells recovered from lymph nodes after 30 days posttransfer. *Left column* shows expression of CD45RB on slowly dividing cells (CFSE$^+$ CD4$^+$). *Right column* illustrates expression of CD45RB on all recovered Thy-1.1$^+$ CD4$^+$ lymphocytes.

In the first experiment, we isolated CD4$^+$ T cells from β_2-microglobulin-deficient wild-type mice (A^{b}wtβ_2m$^-$) or mice expressing only A^{b}Ep complex (A^{b}EpIi$^-\beta_2$m$^-$), labeled them with CSFE, and separately adoptively transferred them into sublethally irradiated A^{b}EpIi$^-\beta_2$m$^-$ recipients. Two days after transfer, bright CD4$^+$CSFE$^+$ T cells from both types of donors were present at significant amounts in recipients expressing A^{b}Ep complex (Fig. 4). In contrast, 2 wk after transfer, most of CSFE$^+$CD4$^+$ T cells from the A^{b}EpIi$^-\beta_2$m$^-$ donors were proliferating and declining their CSFE staining, while CD4$^+$ T cells from the A^{b}wtβ_2m$^-$ donors divided once or not at all. Four weeks after transfer, multiple divisions of A^{b}EpIi$^-\beta_2$m$^-$-derived CD4$^+$ T cells were even more pronounced. These results implied that the A^{b}Ep complex supports the expansion of self-selected CD4$^+$ T cells, but is unable to support survival of CD4$^+$ T cells that developed in mice expressing A^b molecules bound with many self peptides.

In the second experiment, CD4$^+$ T cells isolated from wild-type B6.PL mice (Thy-1.1$^+$) and A^{b}EpIi$^-\beta_2$m$^-$ (Thy-1.1$^-$) mice were mixed in equal numbers, labeled with CFSE, and transferred into the recipient mice. In these experiments, A^{b-} mice were lethally irradiated and reconstituted with bone marrow cells from A^{b}EpIi$^-$ mice. Such radiation chimeras express a single A^{b}Ep complex in the periphery, but have very few CD4$^+$ T cells because they lack expression of A^b on thymic epithelium. Four weeks after reconstitution, we injected these chimeras with a mixture of 5×10^6 CFSE-labeled CD4$^+$ T cells from wild-type and A^{b}EpIi$^-\beta_2$m$^-$ mice and followed their fate in vivo. As shown in Fig. 5, virtually all CD4$^+$ T cells were CFSE positive 2 days after transfer, whereas on day 9, the majority of transferred cells were already CFSE negative, regardless of their origin. However, in less than 3 wk, the population of transferred CD4$^+$Thy-1.1$^-$ T cells almost completely lost the CFSE staining due to proliferation, while the majority of CD4$^+$Thy-1.1$^+$ T cells remained CFSE positive. When the total number of transferred CD4$^+$ T cells was calculated, we found that the CD4$^+$ T cells derived from A^{b}EpIi$^-$ mice expanded several times, while CD4$^+$ T cells derived from wild-type mice were almost completely extinct (Fig. 5*B*). In previous experiments, the same wild-type CD4$^+$ T cells expanded without restraint if transferred into mice expressing A^{b}wt in the periphery (Figs. 2 and 3), proving that they can proliferate in the appropriate MHC/peptide environment. Hence, we concluded that to survive and expand, naive CD4$^+$ T cells favor contact with the MHC/peptide complexes that were encountered during their thymic selection.

Activated CD4$^+$ T cells can persist for weeks in the absence of the selecting class II MHC/peptide ligand, but preferentially expand in its presence

The survival of naive and Ag-experienced T cells in the periphery is regulated differently, and both populations are believed to occupy nonoverlapping ecological niches (26). Memory CD8$^+$ T cells transferred into host with nonrestricting class I MHC survived for the extended period of time (19), but decayed in 1 wk in D^{b-}K$^{b-}\beta_2$m$^-$-deficient mice (27), implying that effector/memory T cells may not tightly depend on the expression of a particular type of MHC. Similarly, it was reported that in vitro generated effector CD4$^+$ T cells transferred into mice that do not express specific Ag survived for several weeks (7, 28). However, whether activated CD4$^+$ T cells require particular self peptides bound to MHC to persist, as naive cells do, has not been investigated. To assess whether self peptides bound to A^b influence the survival of activated CD4$^+$ T cells, we transferred in vitro activated CD4$^+$ Thy-1.1$^+$ T cells into sublethally irradiated recipients that lack A^b or express A^b bound with one or with many peptides. The effector CD4$^+$ Thy-1.1$^+$ T cells were generated from lymph node-derived naive CD4$^+$ T cells during 6 days of incubation in the presence of IL-2 and immobilized mAb specific for $\alpha\beta$TCR. Activated CD4$^+$ Thy-1.1$^+$ T cells were then labeled with CFSE, and adoptively

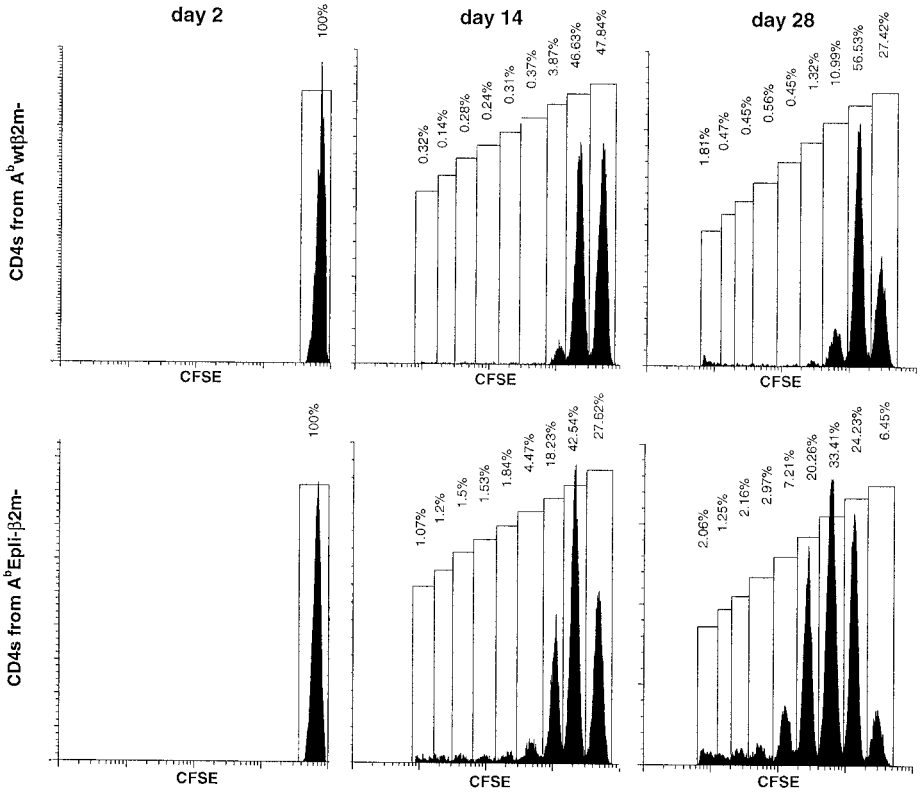

FIGURE 4. In the periphery, the A^bEp complex supports the survival of self-selected naive $CD4^+$ T cells, but not naive $CD4^+$ T cells selected on many A^b/self peptide complexes from $A^bwt\beta_2m^-$ mice. A total of 3×10^6 CSFE-labeled $CD4^+$ T cells isolated either from $A^bEpIi^-\beta_2m^-$ or from $A^bwt\beta_2m^-$ mice were separately adoptively transferred into sublethally irradiated $A^bEpIi^-\beta_2m^-$ recipients, and the number of divisions was detected on days 2, 14, and 28 after transfer. Histograms show only $CD4^+$ $CSFE^+$ cells. The average number of recovered $CD4^+$ $CSFE^+$ cells was 4.58×10^6 from the $A^bEpIi^-\beta_2m^-$ donor and 1.17×10^6 from the $A^bwt\beta_2m^-$ donor.

transferred into sublethally irradiated recipients. As shown in Fig. 6*A*, 2 days after transfer, all $CD4^+$ Thy-1.1^+ cells remain CFSE positive in all groups of recipients. However, when recipients were sacrificed 30 days after transfer, the majority of $CD4^+$ Thy-1.1^+ T cells recovered from wild-type mice were CFSE negative, in contrast to two other recipients in which more than one-half of the transferred cells retained the dye. As shown in Fig. 6*B*, the number of recovered $CD4^+$ Thy-1.1^+ T cells increased in wild-type recipients concurrent with the staining data, implying that to expand, wild-type derived effector $CD4^+$ T cells may favor expression of multiple endogenous peptides bound to A^b. The number of $CD4^+$ Thy-1.1^+ T cells recovered from the two other recipients was close to the number transferred initially, and one-half of them were CFSE negative, indicating that at least a portion of transferred effector $CD4^+$ T cells expanded regardless of the presence or absence of class II MHC.

To determine whether A^bEp complex can extend survival of self-selected $CD4^+$ T cells after they have been activated, we isolated $CD4^+$ T cells from $A^bEpIi^-\beta_2m^-$ (Thy-1.1^-) or from wild-type mice (Thy-1.1^+), activated them in vitro, labeled them with CFSE, and adoptively transferred them into lethally irradiated MHC-deficient mice reconstituted with bone marrow from A^bEpIi^- mice. These chimeric mice have almost no $CD4^+$ T cells and express single A^bEp complex in the periphery. As shown in Fig. 7*A*, 2 days after transfer, Thy-1.1^- and Thy-1.1^+ $CD4^+$ T cells were all CFSE positive. However, on day 9, 31% of transferred $CD4^+$ Thy-1.1^- cells, vs 83% of Thy-1.1^+ T cells, retained the dye. Similar proportions of CFSE-positive and CFSE-negative cells were found after 21 days, when recipients were sacrificed and the number of recovered cells was calculated (Fig. 7*B*). Activated $CD4^+$ Thy-1.1^- cells from $A^bEpIi^-\beta_2m^-$ mice almost doubled, whereas the wild-type derived effector $CD4^+$ T cells did not decay, but also did not expand. Therefore, we conclude that low affinity contact with self class II MHC/peptide ligand(s) may be required by some of activated $CD4^+$ T cells.

Discussion

During intrathymic development, there is an obligatory requirement that all immature T cells have to engage with low affinity self MHC/peptide complex(es) present on thymic stromal cells to survive. There is much experimental evidence to indicate that an enormous diversity of the natural repertoire of expressed TCRs requires many self peptides to be involved in the processes of thymic selection (29–32). Whether a similar demand is mandatory for the sustained survival of peripheral T cells poised for antigenic challenge remains an open question. Two recent reports showed that wild-type $CD4^+$ T cells with native or transgenic TCRs vanished when adoptively transferred into sublethally irradiated H2-DM-deficient (H2-DM^-) mice expressing A^b molecules predominantly bound with class II-associated Ii chain peptide (24, 25). These results implied that naive $CD4^+$ T cells continue to specifically recognize self peptides bound to A^b molecules in wild-type, but not in H2-DM^- mice. However, the configuration of A^b bound with peptides is different in H2-DM^- and wild-type mice, and $CD4^+$ T cells recognize the A^b in H2-DM^- or wild-type mice differently (33). For that reason, $CD4^+$ T cells from wild-type mice may recognize poorly not only few peptides bound to A^b in the absence of H2-DM, but also A^b molecules themselves.

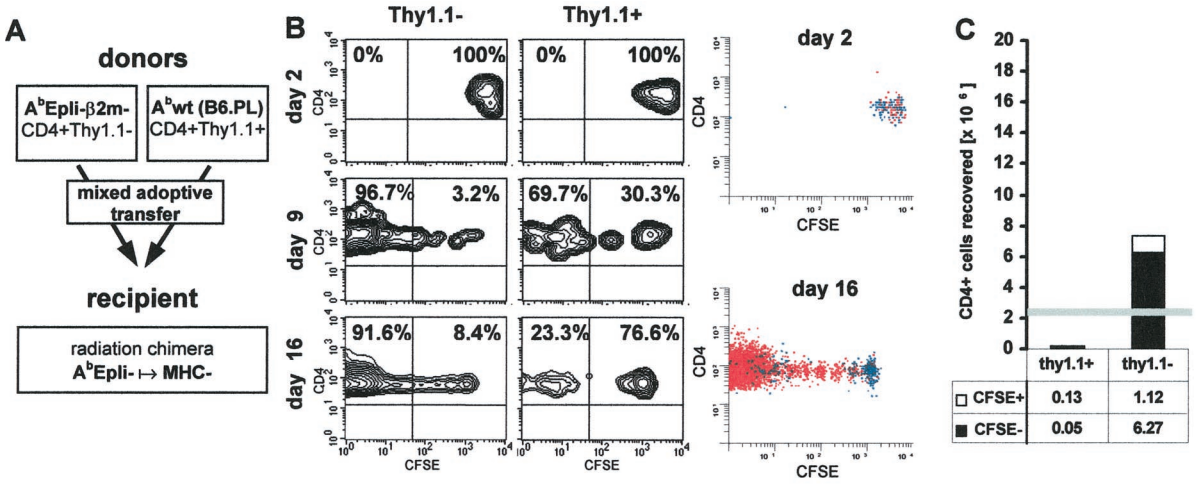

FIGURE 5. Constant exposure to selecting ligand is required for survival and expansion of adoptively transferred naive CD4$^+$ lymphocytes in the periphery. *A*, A^{b}EpIi$^-$→MHC$^-$ chimeras were injected with a mixture containing equal numbers (2.5×10^6) of CFSE-labeled CD4$^+$ lymphocytes selected on MHC class II occupied by many peptides from B6.PL (Thy-1.1$^+$) and selected on single MHC II/peptide complex from A^{b}EpIi$^-\beta_2$m$^-$ animals (Thy-1.1$^-$). Adoptively transferred cells could be readily distinguished by FACS analysis on the basis of their Thy-1.1 expression. *B*, Contour plots depict divisions in the compartment of cells selected on A^{b}Ep complex (Thy-1.1$^-$, *left column*) and cells selected on A^{b}wt (Thy-1.1$^+$, *right column*). To illustrate quantitative differences in survival of transferred cells, data on days 2 and 16 are shown as color dot plots using multicolor gating. Blue dots depict cells from gate CD4$^+$ Thy-1.1$^+$; red dots represent events from gate CD4$^+$ Thy-1.1$^-$. Results from one of six experiments are shown. *C*, Total numbers of CFSE$^+$ and CFSE$^-$ cells in the compartment of Thy-1.1$^+$ and Thy-1.1$^-$ cells recovered from peripheral lymphatic organs of animals shown in *A*. Numeric value ($\times 10^6$) is shown in tables. Grey lines represent number of cells transferred initially.

In this study, we show that the majority of naive CD4$^+$ T cells selected in wild-type mice and adoptively transferred into mice expressing a single class II MHC/peptide complex quickly diminished, with similar kinetics as reported for CD4$^+$ T cells seeded into mice that lack class II MHC molecules (12). Hence, this result implies that CD4$^+$ T cells use their TCRs to sense self peptides bound to MHC molecules not only during selection in thymus, but also in the secondary lymphoid organs, where these interactions maintain prolonged survival of the T cells.

What are the features of the self peptides that uphold viability of naive T cells in the periphery and what is their relationship to the self peptides that select the same T cells in the thymus? One answer to this question is that selecting MHC/peptide may be one of the MHC/peptide complexes able to maintain the persistence of

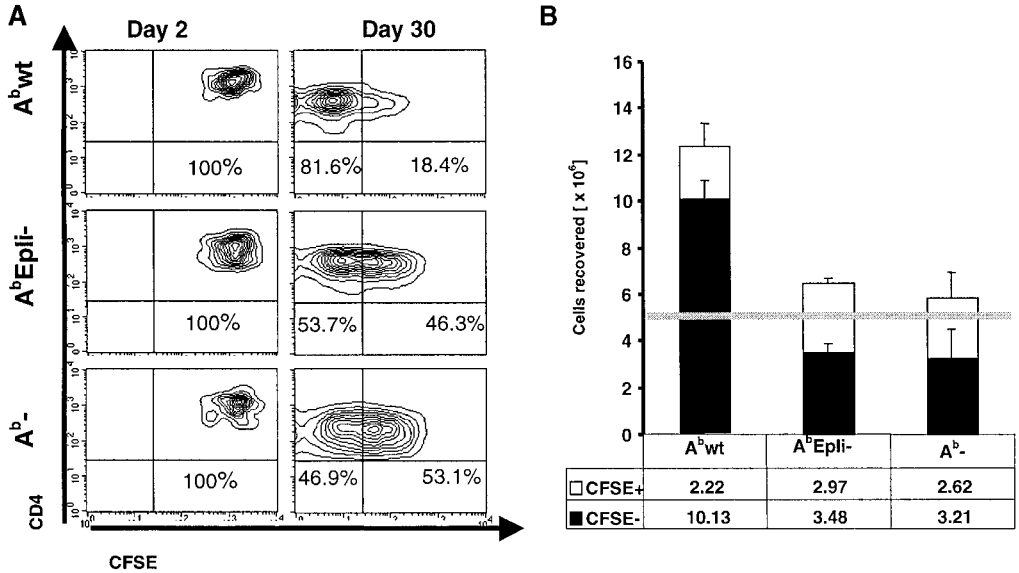

FIGURE 6. Preactivated CD4$^+$ cells from A^{b}wt donors survive and proliferate regardless of the presence of MHC class II. However, they actively expand only if exposed to A^{b}wt. Purified CD4$^+$ cells from B6.PL (Thy-1.1$^+$) donors were incubated with anti-TCR mAb and IL-2 for 6 days, they were subsequently labeled with CFSE, and adoptively transferred into 600 R irradiated recipients displaying various MHC class II environments: A^{b}wt (CD4$^-$), A^{b}EpIi$^-$, and A^{b-}. *A*, Contour plots show proliferation of transferred cells on days 2 and 30. *B*, Total numbers of cells recovered from peripheral lymphatic organs 30 days after transfer with numeric values ($\times 10^6$) presented in the table. Four to six animals per each group were used. Grey lines visualize number of cells transferred initially (5×10^6).

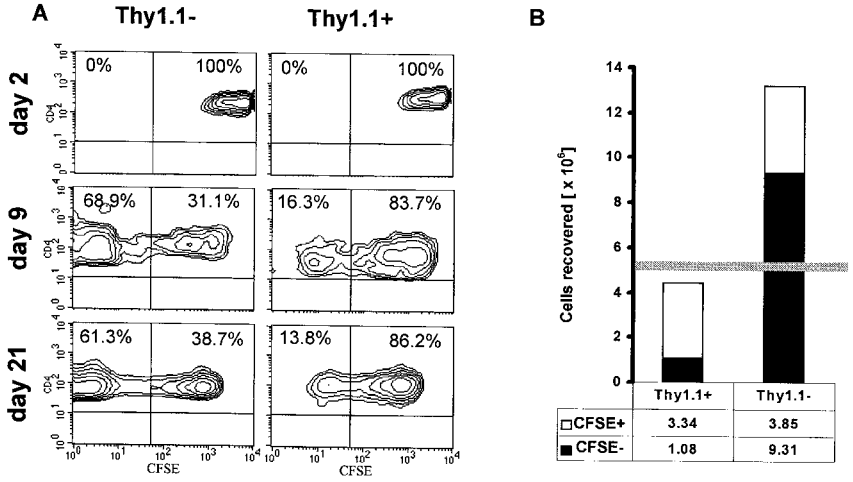

FIGURE 7. Previously activated CD4$^+$ cells from A^{b}EpIi$^-$ mice expand when exposed to selecting MHC II/peptide complex in the periphery, while cells selected on A^{b}wt survive in constant numbers and proliferate slowly. Equal numbers (5×10^6) of in vitro activated CD4$^+$ cells from B6.PL (Thy-1.1$^+$) and A^{b}EpIi$^-\beta_2$m$^-$ (Thy-1.1$^-$) donors were transferred into A^{b}EpIi$^-\rightarrow$ MHC$^-$ radiation chimeras. Representative results from one of three experiments are shown. *A*, Contour plots depict proliferation of Thy-1.1$^-$ (*left column*) and Thy-1.1$^+$ (*right column*) CD4$^+$ lymphocytes. *B*, Total number of Thy-1.1$^+$ and Thy-1.1$^-$ CD4$^+$ T cells recovered, with proportion of cells retaining fluorescent dye (CFSE$^+$). Table shows numeric values ($\times 10^6$).

these T cells in the periphery. The similarity of the MHC/peptide(s) ligand responsible for the selection and maintenance of the clonotypic TCR has been implicated in several reports, but experimental data proving this assumption have not yet been convincingly presented (24, 25, 33, 34). In our experiments, transfer of naive CD4$^+$ T cells from A^{b}EpIi$^-$ or wild-type mice into lethally irradiated A^{b-} mice reconstituted with bone marrow from A^{b}EpIi$^-$ mice resulted in the preferential expansion of only CD4$^+$ T cells from A^{b}EpIi$^-$ mice. In contrast, wild-type derived naive CD4$^+$ T cells disappeared at large by 3 wk after transfer, indicating that A^{b}Ep complex supports primarily the survival of CD4$^+$ T cells selected by this complex. The kinetics of the decay of wild-type CD4$^+$ T cells was slightly accelerated in the presence of CD4$^+$ T cells derived from A^{b}EpIi$^-$ mice, implying that competition between peripheral T cells may also have some influence on their survival. We are currently testing this idea using CD4$^+$ T cells with clonotypic TCRs positively selected by different class II MHC/peptide complexes. Admittedly, a single class II MHC/peptide complex does not match the high expression level of A^b molecules bound with self peptides present in wild-type mice. Therefore, one may argue that if the survival signal for naive CD4$^+$ T cells is calibrated by thymic selection, discrepancy in the expression level of A^b encountered by the same T cell in the thymus or periphery may result in its shortened survival (35) However, naive CD4$^+$ Thy-1.1$^+$ T cells exposed in vivo to low levels of transgenic A^b loaded with endogenous peptides in the presence of Ii did not lead to the extinction of transferred T cells for 4 wk after transfer. Hence, it seems that the low level of transgenic A^b is sufficient to maintain the survival of naive wild-type CD4$^+$ T cells only if bound with a diverse spectrum of self-derived peptides. Limited survival of wild-type CD4$^+$ T cells transferred into the A^{b}EpIi$^-$ mice was also not a result of adverse rejection mediated by NK cells, because hosts were compromised by sublethal irradiation before transfer and some of transferred CFSE-positive T cells remained detected in A^{b}EpIi$^-$ mice after 5 wk. Experimental parameters such as the dose of irradiation, number of transferred cells, and duration of the experiment are critical in revealing the proliferation of transferred CD4$^+$ T cells in recipients expressing class II MHC bound with low affinity peptide ligand. Hence, the differences in these settings may be responsible for the distinct conclusion obtained in the similar studies (34). In this study, to show that naive CD4$^+$ T cells require low avidity contacts with self MHC/peptide complexes to survive in the periphery, we transferred 3×10^6 of naive CD4$^+$ T cells and followed their gradual expansion for several weeks. This length of time was required because naive T cells transferred into their original environment divide slowly.

Expansion of naive CD4$^+$ T cells in the suitable in vivo environment does not entirely rely on the continuous subtle contacts between TCRs and self MHC/peptide complexes. To survive, adoptively transferred naive T cells require space prepared by depletion of the residual CD4$^+$ T cell via irradiation or genetic manipulation (25). Moreover, some surface and intracellular molecules have been found differentially expressed in the naive and memory peripheral T cells, and the pools of naive and memory T cells are independently restrained by unknown homeostatic mechanisms (36, 37). Our results imply that activation of peripheral CD4$^+$ T cell via TCR gradually diminishes their requirement for the low avidity interactions with self MHC/peptide complexes, and supports the experiments in which produced in vitro CD4$^+$ memory T cells survived in the absence of class II MHC and Ag (7).

It is unknown why the effector CD4$^+$ T cells that have already been activated via TCR continue to recognize self MHC/peptide ligands, but recently it became clear that several divisions of these cells are required to convert a portion of them into memory T cells (38, 39). In addition, effector CD4$^+$ T cells can persist in vivo for the extended period of time with quantitatively and qualitatively distinct pattern of tyrosine phosphorylation (40). Our experiments indicate that the enhanced proliferation of activated CD4$^+$ T cells occurred in the presence of specific self MHC/peptide complexes in the periphery. Therefore, the ability of effector CD4$^+$ T cells to recognize self MHC/peptide complexes with low affinity may allow them to divide and further differentiate, even in the limited access to Ag.

An experiment in which CD4$^+$ T cells selected by A^{b}Ep are transferred into a recipient expressing in the periphery wild-type A^b bound with many self peptides could not be performed because the majority of CD4$^+$ T cells from A^{b}EpIi$^-$ mice are not tolerant to wild-type peptides. However, in the lethally irradiated A^{b}EpIi$^-$ mice reconstituted with fetal liver cells from wild-type mice, after an initial drop in CD4$^+$ T cell number due to extensive negative selection, a pool of peripheral CD4$^+$ T cells with TCRs encoding

various Ag specificities remained unchanged for several months (41). This result indicated that some of the T cells selected by A^bEp complex may cross-react with low affinity to self-derived peptides, subtly enough to avoid negative selection, but adequate to survive in the periphery.

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