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Altered Selection of CD4$^+$ T Cells by Class II MHC Bound with Dominant and Low Abundance Self-Peptides

Anna Gaszewska-Mastalarz, Pawel Muranski, Bartosz Chmielowski, Piotr Kraj, and Leszek Ignatowicz

We have investigated the development of CD4$^+$ T cells in mice expressing low levels of transgenic class II MHC molecules (A$b$) preoccupied with covalent peptide (Ep), which in the presence of invariant chain (Ii) is extensively cleaved and replaced with self-derived peptides. In these mice, the transgenic A$b$ molecules, bound with predominant peptide (Ep) and with multiple self-peptides, selected more CD4$^+$ T cells than A$b$/self-peptide complexes expressed in wild-type mice. The enhanced outcome of thymic selection was a result of impaired negative selection, rather than more efficient positive selection by an overall lowered abundance of self-derived A$b$/peptide complexes. Peripheral CD4$^+$ T cells in the A$b$EpIi$^+$ mice had memory phenotype, often followed by polyclonal activation of B cells. The A$b$EpIi$^+$ mice preserved their good health and had a normal life span despite the profound number of activated CD4$^+$ T cells and B cells in peripheral lymphoid organs, moderate hypergammaglobulinemia, and deposited complexes in the kidneys. We propose that CD4$^+$ T cells positively selected due to low avidity for high abundant A$b$Ep complex avoid negative selection on A$b$ molecules loaded with low abundant peptides and become self-reactive in the peripheral lymphoid organs. The Journal of Immunology, 2000, 165: 6099–6106.

Positive selection rescues T cells with TCRs that bind self-MHC/peptide complexes expressed on thymic epithelial cells with low avidity (1–3). Thymocytes with TCRs that do not bind self-MHC/peptide complexes die neglected, and thymocytes that recognize these complexes with high avidity are deleted by negative selection (4–6). Naive T cells that leave the thymus migrate to the periphery where they must perceive self-MHC/peptide complexes to survive and expand (7, 8). Analysis of T cell repertoires in genetically manipulated mice expressing class II MHC molecules exclusively on thymic stromal cells or in association with one peptide clearly showed that positive selection indiscriminately generates a large number of potentially self-destructive T cells. On the average, one-half to three-quarters of positively selected T cells are believed to be negatively selected, implying that most T cells expressing TCRs that can bind MHC in the thymus (9, 10). Because the conditions for positive selection and survival bias naive T cells to continuously perceive self-MHC/peptide complexes, there is an increased risk that some of these cells may elicit undesirable reactions against host tissue. The autoreactive T cells often express TCRs with low avidity for self-MHC/peptide complexes, indicating that regardless of the high sensitivity of negative selection, self-destructive T cells leak to the periphery (11, 12). Hence, it is essential to determine how an abundance of the specific MHC/self-peptide complexes and their individual properties may diminish the effectiveness of negative selection.

It is imaginable that physiological expression of MHC evolved to display an optimum of different peptides to T cells. An average APC expresses about $10^5$–$10^6$ class II MHC molecules bound with $10^3$–$10^4$ different peptides derived from self and non-self proteins. The distribution of peptides across class II MHC molecules varies; some peptides capture 10% of total class II MHC molecules and are displayed to CD4$^+$ T cells at high abundance, while other peptides bind only a few MHC molecules per cell and are presented at low abundance (13, 14). The immune system can augment presentation of low abundant MHC/peptide complexes to T cells. For example, infrequently expressed MHC/peptide complexes may selectively relocate, upon TCR/coreceptor engagement, into the cell-cell contact site called the immunological synapse to enhance their display to T cells. It has been also proposed that inside the immune synapse, few specific MHC/peptide complexes serially engage multiple αβTCRs and trigger the T cell activation signaling pathway (15). It is likely that both low and high abundant peptides bound to MHC influence T cell development, as shown by different studies conducted with T cells selected in mutant mice expressing class II MHC molecules occupied with dominant peptide. Whereas one study emphasized the importance of low abundant peptides bound to class II MHC on thymic selection of CD4$^+$ T cells in these mice, the other studies found evidence for the imprint of dominantly expressed peptide that led to the selection of CD4$^+$ T cells with altered self-reactivity (14, 16).

Here, we have examined the development of CD4$^+$ T cells in mice expressing transgenic A$b$-chain covalently associated with Etr$_{2–88}$ Peptide in the absence of endogenously expressed A$b$-chain (9). In these mice expression of class A$b$ molecules is reduced 10-fold on all bone marrow-derived APCs compared with wild-type mice, and the covalently attached peptide is replaced at large with endogenously derived peptides due to the presence of...
invariant chain (Ii). In these mice the Aβ molecules occupied with dominant Ep peptide and low abundant, multiple self-peptides selected many CD4+ T cells. The majority of these CD4+ T cells expressed activation markers, but did not cause damage to host tissues. We propose that these CD4+ T cells were positively selected on the dominant AβEp complex and acquired a memory phenotype due to diminished negative selection and cross-reactivity for low abundant peptides.

**Materials and Methods**

**Animals**

The C57BL/6 mice (Aβ wt) and mice deficient in endogenous TCR α-chain (TCRα−) were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice deficient for the wild-type Aβ-β-chain (Aβ−) were provided by D. Mathis (Harvard Medical School, Boston, MA). Mice transgenic for the AβEp construct and deficient for the wild-type Aβ-β-chain (AβEp−) were generated at the National Jewish Medical and Research Center (Denver, CO) as previously described (9) and backcrossed on the C57/BL6 background. Mice transgenic for αβTCR specific for Aβ and pigeon cytochrome c (PCC45-88) Peptide were generated in our laboratory (P. Kraj, unpublished observations) and crossed with mice transgenic for AβEp and devoid of endogenous Aβ-β-chain and TCR α-chain (AβEp−/α−). All mice were further bred in the animal care facility at the Medical College of Georgia (Augusta, GA).

Chimeric mice were generated by irradiation of 6-wk-old animals (1100 rad) followed by i.v. reconstitution with 5 × 10^9 T cell-depleted bone marrow. Chimeras were analyzed at least 8–10 wk later.

**Cell staining**

Cells were stained for CD4 (GK1.5), CD8 (53-6.7), CD69 (H1.2F3), CD44 (IM7), CD62L (MEL14), CD45RB (16A), and different Vβ segments as previously described (17). The fluorescein- and PE-labeled Abs were purchased from Becton Dickinson (San Diego, CA) or were made in our laboratory. Briefly, cells were suspended in staining buffer (balanced salt solution, BSS), 0.1% sodium azide, and 2% FBS and were incubated for 30 min at 4°C with the Abs of interest in the presence of 10% normal mouse serum and 10% anti-Fc receptor mAb (2.4.G2). Cells were then washed three times with staining buffer and analyzed using a FACScalibur instrument (Becton Dickinson).

**Tissue staining**

For detection of IgG deposits in kidney glomeruli, kidneys were embedded in OCT compound (Sakura Finetek, Torrance, CA) and snap-frozen. Five-μm sections were air-dried and fixed with cold acetone for 10 min. These cryosections were and stained with FITC-labeled goat anti-mouse IgG for 1 h at room temperature. Sections were analyzed, and photographs were taken using a Axiophot fluorescent microscope (Karl Zeiss, Thornwood, NY) and video camera (Photometrics, Tuscon, AZ).

**Ab assays**

Total levels of serum IgM and IgG subclasses were determined by ELISA using alkaline phosphatase-labeled goat Abs specific for mouse Ig classes and subclasses (Southern Biotechnology Associates, Birmingham, AL). The Ig concentrations were determined by referring to standard curves obtained with known concentrations of mouse Ig (Southern Biotechnology Associates).

**Proliferation assays**

The responses of CD4+ T cells selected in AβEp+ mice for Aβ self-peptide complexes were tested by incubating purified CD4+ T cells with irradiated splenocytes (3000 rad). Lymph node CD4+ T cells from AβEp+ were purified by complement depletion as described using cytotoxic cocktail 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 25.6-35 and BP107.2.2), anti-CD45 (clone B220), and anti-J11D (clone J11D.2) (17). The proliferative response was measured on the third day using an 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay (18).

**Results**

**Mice expressing fewer Aβ molecules bound with various self-derived peptides select more CD4+ T cells than wild-type mice**

We have recently described transgenic mice that express a β-chain of mouse class II MHC molecule (Aβ) covalently linked with the single E552-68 peptide in the absence of endogenous Aββ and invariant chain (9). Due to the exclusive occupancy of all detectable Aβ molecules with one covalent peptide, these mice have a severely compromised number of CD4+ T cells in the thymus and periphery. However, the number of CD4+ T cells is high in mice that express the AβEp transgene in the absence of endogenous Aββ chain but with normal expression of the invariant chain (AβEp+ mice). The presence of the invariant chain leads to cleavage of the covalent peptide, which is replaced with a diverse set of self-peptides (21). In AβEp+ mice, around 30% of the Aβ molecules are occupied with Ep, while the rest are bound with low abundant, self-derived peptides. Interestingly, the number of selected CD4+ T cells in the thymus or periphery in the AβEp+ mice was roughly two times higher than the number of CD4+ T cells found in wild-type mice (Fig. 1A). The repertoire of TCRs expressed on CD4+ T cells found in the AβEp+ mice was polyclonal, and the frequencies of Vβ segments used in these TCRs were similar to the Vβ pattern recorded for wild-type mice (Fig. 1B). To determine why the reduced number of Aβ peptide complexes facilitates selection of a higher number of CD4+ T cells in vivo, we examined the expression level of Aβ molecules on different types of APCs in AβEp+ mice. Thymic epithelial cells from AβEp+ mice vs wild-type mice had two times less Aβ expressed, while splenic APCs had 10 times fewer Aβ molecules expressed than the respective subpopulation of APCs isolated from wild-type mice. The Aβ peptide complexes present in APCs from AβEp+ mice stained positively with mAbs specific for Aβ, including ones that depend on the expression of particular endogenously derived peptides (Fig. 2A). In addition, three T cell hybridomas specific for Aβ and endogenously derived peptides secreted IL-2 after overnight incubation with spleen cells derived from AβEp+ mice (Fig. 2B). These results implied that in AβEp+ mice the Aβ molecules are occupied with dominant Ep and low abundant self-peptides.

The greater number of CD4+ T cells found in AβEp+ mice could be a result of enhanced positive selection due to reduced expression of Aβ peptide complexes on thymic epithelium. Positive selection is mediated by low avidity interactions between TCR and self-MHC/peptide complexes, and there are experiments implying that positive selection uses low abundant class II MHC/peptide complexes more favorably than high abundant class II MHC/peptide complexes. Alternatively endogenous peptides in AβEp+ mice have to compete with cleaved Ep for binding to Aβ, which may bias the repertoire of self-peptides toward ones that...
Finally, in A\\textsuperscript{Epli} mice, the A\textsuperscript{b} molecules may be poorly expressed on thymic macrophages and dendritic cells, but are only moderately reduced on thymic epithelial cells. This divergence in expression of A\textsuperscript{b}/peptide complexes on different thymic stromal cells might also cause alterations in the T cell selection processes. Hence, to compare the efficiency of positive selection of CD4\textsuperscript{+} T cells on thymic epithelial cells in A\textsuperscript{Epli} or wild-type mice, we lethally irradiated both types of mice and reconstituted them with bone marrow cells from class II MHC-deficient mice (A\textsuperscript{b}-/-). In these chimeras, positive selection proceeded on thymic epithelium of A\textsuperscript{Epli} or wild-type hosts in the absence of negative selection on hemopoietic APCs. As shown in Fig. 3, the numbers of CD4\textsuperscript{+} T cells positively selected by wild-type and A\textsuperscript{Epli} epithelium were comparable. This experiment indicated that A\textsuperscript{b}/peptide complexes expressed in A\textsuperscript{Epli} mice do not positively select more CD4\textsuperscript{+} T cells than they do in wild-type mice.

**Impaired negative selection in A\textsuperscript{Epli} mice results in an increased number of CD4\textsuperscript{+} T cells in the thymus**

In A\textsuperscript{Epli} mice, reduction in A\textsuperscript{b} expression was greatest on all hemopoietic APCs. Therefore, we next examined whether the negative selection in these mice proceeds with the same efficiency as in wild-type mice. For that purpose, we lethally irradiated A\textsuperscript{Epli} mice and reconstituted them with bone marrow from either A\textsuperscript{Epli} or A\textsuperscript{wt} mice or a mixture of both bone marrows. As shown in Fig. 4A, only mice reconstituted with bone marrow from the autologous donor had an elevated number of CD4\textsuperscript{+} T cells in the thymus and the peripheral lymphoid organs. Chimeras with thymic epithelium expressing low levels of transgenic A\textsuperscript{b}/peptide complexes, but reconstituted with wild-type bone marrow, had a normal number of CD4\textsuperscript{+} T cells. Also, the A\textsuperscript{Epli} recipients that received mixed A\textsuperscript{Epli}/A\textsuperscript{wt} bone marrow had a normal number of CD4\textsuperscript{+} T cells in the thymus, implying that selected CD4\textsuperscript{+} T cells do not expand in the medulla upon exposure to altered transgenic A\textsuperscript{b}/peptide complexes. Similarly, the CD4/CD8 T cell ratio in the thymus or peripheral lymph nodes was 2 times higher only in recipient mice reconstituted with autologous bone marrow and not in the two other recipients (Fig. 4B). The total number of CD4\textsuperscript{+} T cells found in the periphery of A\textsuperscript{Epli} \rightarrow A\textsuperscript{Epli} chimeric mice was nearly three times higher than the number of CD4\textsuperscript{+} T cells found in the two other chimeras (data not shown). Collectively, these estimates imply that in A\textsuperscript{Epli} mice some CD4\textsuperscript{+} T cells eligible for negative selection avoid deletion and proceed to the periphery.

**Accumulation of activated CD4\textsuperscript{+} T cells in the peripheral lymphoid organs in mice coexpressing dominant A\textsuperscript{Ep} complex and A\textsuperscript{b} bound with low abundant peptides**

Experiments described in the previous section implied that in A\textsuperscript{Epli} mice, negative selection is partially impaired, allowing more CD4\textsuperscript{+} T cells to depart to the periphery. To test whether peripheral CD4\textsuperscript{+} T cells in A\textsuperscript{Epli} mice have a naive or activated phenotype, we analyzed the expression of activation markers on this population of cells. As shown in Fig. 5A, far more CD4\textsuperscript{+} T cells in A\textsuperscript{Epli} than in wild-type animals expressed CD69 and CD44 molecules, while fewer expressed high levels of CD62L and CD27 molecules. This demonstrates that activated CD4\textsuperscript{+} T cells in A\textsuperscript{Epli} mice are positively selected in the thymus, implying that selected CD4\textsuperscript{+} T cells do not expand in the medulla upon exposure to altered transgenic A\textsuperscript{b}/peptide complexes. Similarly, the CD4/CD8 T cell ratio in the thymus or peripheral lymph nodes was 2 times higher only in recipient mice reconstituted with autologous bone marrow and not in the two other recipients (Fig. 4B). The total number of CD4\textsuperscript{+} T cells found in the periphery of A\textsuperscript{Epli} \rightarrow A\textsuperscript{Epli} chimeric mice was nearly three times higher than the number of CD4\textsuperscript{+} T cells found in the two other chimeras (data not shown). Collectively, these estimates imply that in A\textsuperscript{Epli} mice some CD4\textsuperscript{+} T cells eligible for negative selection avoid deletion and proceed to the periphery.
in A<sup>EpIi</sup><sup>+</sup> mice was regardless of TCR specificity, we crossed these mice with mice that exclusively express transgenic TCRs specific for A<sup>b</sup> and foreign PCC<sub>43–58</sub> peptide in the absence of endogenous TCR α-chains. Even though T cells bearing this transgenic αβTCR were selected toward the CD4<sup>+</sup> lineage in both A<sup>b</sup> wild-type and A<sup>EpIi</sup><sup>+</sup> mice, these CD4<sup>+</sup> T cells did not acquire an activated phenotype (Fig. 6). Therefore, in A<sup>EpIi</sup><sup>+</sup> mice the CD4<sup>+</sup> T cells have to express αβTCRs with multiple specificities to get activated.

Peripheral CD4<sup>+</sup> T cells from A<sup>EpIi</sup><sup>+</sup> mice mediate bone marrow rejection in lethally irradiated recipients

Despite the significant number of CD4<sup>+</sup> T cells with an activated phenotype that are found in A<sup>EpIi</sup><sup>+</sup> mice, these mice retained good health throughout their lifetimes. The only visible morphological abnormalities noted among these mice were an increased rate of splenomegaly in their third to fourth decade of life and Ab deposits in the kidneys (see below). Moreover, when isolated...

**FIGURE 2.** In A<sup>EpIi</sup><sup>+</sup> mice, a reduced number of A<sup>b</sup> molecules are bound with dominant Ep peptide and low abundant, self-derived peptides. A. Thymic epithelial cells and splenic B cells in A<sup>EpIi</sup><sup>+</sup>, A<sup>wt</sup>, and A<sup>b</sup> mice were identified by staining with BP-1 PE and B220 PE Abs, respectively. These cells were subsequently stained with various Abs (Y3P, YAe, 30-2, 25-9-17S) specific for A<sup>b</sup>. The level of A<sup>b</sup> in A<sup>EpIi</sup><sup>+</sup> vs A<sup>wt</sup> mice on thymic epithelial cells or splenic B cells was reduced 2- or 10-fold, respectively. B. In A<sup>EpIi</sup><sup>+</sup> mice, the A<sup>b</sup> is bound with endogenous peptides that are recognized by specific T cell hybridomas. Hybridomas specific for endogenous peptides, 4.2 specific for β<sub>2</sub>-microglobulin 48–58, 77.1 specific for IgM 377–392, and 7.6 specific for CD22 25–39 and A<sup>b</sup>, were incubated in the presence of different numbers of A<sup>EpIi</sup><sup>+</sup> splenocytes. The level of IL-2 produced was measured by HT-2 assay as previously described (17).
CD4⁺ T cells were cultured with APCs expressing autologous or normal level of Aβ/peptide complexes, no significant proliferation was detected (data not shown).

To determine whether the CD4⁺ T cells found in AβEpIi mice can mediate GvH reaction, we lethally irradiated the AβEpIi mice and reconstituted them with autologous, T cell-depleted bone marrow together with 5 × 10⁶ mature CD4⁺ T cells isolated from Aβ wt, Aβbm12, or AβEpIi mice. Chimeras that received bone marrow together with CD4⁺ T cells from Aβ wt mice survived well over 4 mo, while the ones that received CD4⁺ T cells from Aβbm12 succumbed to GvH disease after 2–3 wk (Fig. 7). In contrast, mice that received bone marrow and CD4⁺ T cells from AβEpIi mice remained healthy for 4 wk, but than all five recipients died during the following week. These results suggested that CD4⁺ T cells transferred from Aβbm12 or AβEpIi mice mediate GvH, which is delayed, probably due to the lower expression level of Aβ/peptide complexes on the AβEpIi bone marrow APCs.

In AβEpIi mice activated B cells cause splenomegaly and secrete Igs that are deposited in the kidneys

CD4⁺ T cells that terminally differentiate toward the Th2 lineage secrete IL-4 and IL-5, which activates naive B cells. During the staining of CD4⁺ T cells for CD25 expression, we noticed that in AβEpIi mice, but not in wild-type mice, there is a separate population of cells bearing this marker. These cells also stained for B220 and CD19, but not for Thy1. Because CD25 is an activation marker also expressed on B cells, this result implied that AβEpIi mice also have an increased number of activated B cells in the peripheral lymphoid organs. We next determined that these activated B cells secrete more Ig than normally found in the serum of wild-type mice. As shown in Fig. 8A, the AβEpIi mice had elevated serum levels of IgG1 and IgG2b, suggesting that polyclonal activation of CD4⁺ T cells also results in minor hyper gammaglobulinemia. However, these Abs were not directed against ssDNA or chromatin, and we found no pathological deposits of ssDNA or chromatin.
autoantibodies in the joints (data not shown). Immunocomplexes were found in the kidneys of 40% of these mice, but the mice did not develop proteinuria. These results imply that a leak of autoreactive CD4\(^+\) T cells, which escaped deletion in the thymus, activates B cells that secrete an increased amount of Ig, leading to early stages of glomerulonephritis (Fig. 8B).

**Discussion**

We have analyzed thymic selection of CD4\(^+\) T cells in mice expressing low levels of transgenic A\(^b\) molecules concurrently occupied with dominant Ep peptide and many low abundant peptides. In these mice the overall expression level of A\(^b\) molecules was only slightly decreased on thymic epithelial cells, but was reduced 10-fold on all thymic and peripheral hemopoietic APCs. Uniquely, CD4\(^+\) T cells were selected in the thymus of these mice more efficiently than in wild-type thymus. Moreover, these CD4\(^+\) T cells were prone to spontaneous activation in the peripheral lymphoid organs. To determine whether thymic selection processes may be responsible for this phenomenon, we made different radiation chimeras expressing the transgenic A\(^b\) molecules on various thymic stromal cells. The quantitative estimates derived from these experiments showed that the decreased expression of A\(^b\) molecules cooccupied with dominant Ep peptide and multiple low abundant self-peptides resulted in impaired negative selection of CD4\(^+\) T cells. The CD4\(^+\) T cells that avoid deletion in the thymus expressed TCRs with low avidity for A\(^b\)/self peptides, and we could not elicit a response in vitro to wild-type APCs expressing A\(^b\)/peptide complexes at a higher level (see below). Additionally, none of the 80 randomly generated CD4\(^+\) T cell hybridomas derived from A\(^b\)EpIi\(^+\) mice produced IL-2 when incubated overnight with wild-type APCs (data not shown). Some of the CD4\(^+\) T cells isolated from A\(^b\)EpIi\(^+\) mice expressed CD25 and intermediate levels of CD45, which may be indicative of the presence of regulatory CD4\(^+\) T cells, an issue that we are currently investigating (22).
that the CD4+ T cells in AβEpII+ mice are functional (data not shown). CD4+ T cells in AβEpII+ mice expressed activation markers such as CD69high, CD44high, CD62Llow, and CD45RBhight only if their αβTCRs were heterogeneous, suggesting that a signal provided by αβTCR recognition of Aβ/peptides is required to activate these cells. B cells are also activated in AβEpII+ mice and secrete more IgG1s. Because B cell activation and Ab isotype switch are driven by the Th2 type of CD4+ T cells, this observation suggests that chronic exposure to Aβ occupied with unknown self-peptides in AβEpII+ mice might bias the terminal differentiation of CD4+ T cells.

The properties of CD4+ T cells found in AβEpII+ mice resemble some of the properties of CD4+ T cells found in mice expressing Aβ/self-peptide complexes only on thymic epithelium (K14 mice) (2). These latter CD4+ T cells, upon adoptive transfer into nonirradiated recipients with wild-type Aβ/self-peptide complexes on hemopoietic APCs, became activated and provoked B cell activation, but did not cause an adverse GvH reaction (23). However, coinjection of autologous bone marrow and CD4+ T cells from K14 mice into lethally irradiated wild-type recipients provoked rapid failure of bone marrow engraftment. Additional experiments in which the original K14 mice were crossed with mice that express few of the transgenic Ab/peptide complexes on dendritic and hemopoietic thymic stromal cells. Consequently, the question arises of which of the CD4+ T cells, those selected on Aβ bound with Ep or those selected on low abundant self-peptides, are spontaneously activated in the peripheral lymphoid organs in these mice. We favor the hypothesis that the AβEp complex selects self-reactive CD4+ T cells that avoid negative selection on the rest of the transgenic Aβ molecules bound with different endogenous peptides. These CD4+ T cells are activated in the peripheral lymphoid organs as they continue to recognize Aβ bound by low abundant, endogenous peptides. With age, the accumulation of CD4+ T cells with memory phenotype is followed by polyclonal activation of B cells. Although the health or life span of the AβEpII+ mice is not compromised, an onset of autoimmune disease requires prolonged avidity maturation of the TCR repertoire, and a few of the Aβ/peptide complexes may be insufficient to drive such selection in the periphery (25).

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Published analysis of the repertoire of TCRs selected by AβEp complex showed that these TCRs frequently recognize Aβ molecules loaded with self-peptides (9). Because the AβEp complex remained dominantly expressed in AβEpII+ mice, this complex might positively select a minor population of CD4+ T cells in these mice. Consequently, the question arises of which of the CD4+ T cells, those selected on Aβ bound with Ep or those selected on low abundant self-peptides, are spontaneously activated in the peripheral lymphoid organs in these mice. We favor the hypothesis that the AβEp complex selects self-reactive CD4+ T cells that avoid negative selection on the rest of the transgenic Aβ molecules bound with different endogenous peptides. These CD4+ T cells are activated in the peripheral lymphoid organs as they continue to recognize Aβ bound by low abundant, endogenous peptides. With age, the accumulation of CD4+ T cells with memory phenotype is followed by polyclonal activation of B cells. Although the health or life span of the AβEpII+ mice is not compromised, an onset of autoimmune disease requires prolonged avidity maturation of the TCR repertoire, and a few of the Aβ/peptide complexes may be insufficient to drive such selection in the periphery (25).

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