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An Incremental Increase in the Complexity of Peptides Bound to Class II MHC Changes the Diversity of Positively Selected \( \alpha\beta \) TCRs

Rafal Pacholczyk, Piotr Kraj, and Leszek Ignatowicz

Positive selection of the normal repertoire of TCRs results from low-avidity interactions with a set of self-peptides bound to the MHC molecules expressed by thymic epithelial cells. The contribution of the individual peptide to positive selection remains a matter of debate. Here, for the first time, we show that two covalent class II MHC-peptide complexes positively select different TCRs expressing a common transgenic TCR\( \beta \)-chain and endogenous TCR\( \alpha\)-chains. Simultaneous expression of both \( \alpha\)-peptide complexes changed the diversity of positively selected TCRs, indicating an additive and possibly synergistic effect of various peptides in this process. The Journal of Immunology, 2001, 166: 2357–2363.

The \( \alpha\beta \) TCR is inherently biased to bind MHC molecules, but only a few immature T cells survive thymic selection (1–3). The majority of immature T cells die by neglect or are actively deleted as potentially self-reactive. Only, if the thymocyte TCR weakly binds self-MHC-peptide complexes expressed on thymic epithelium, the immature T cell is rescued in the process known as positive selection (4, 5). The contribution of individual peptides to thymic selection remains unknown, although a normal repertoire of TCRs was found to be selected only when many wild-type (wt) peptides were bound to MHC on thymic stromal cells. Different peptides bound to MHC may be required to generate nonoverlapping sets of TCRs, which will result in an additive increase in the diversity of the selected TCR repertoire (6). Moreover, peptide diversity may have a synergistic effect on the TCR repertoire by creating an environment where the “gemish” of peptides, rather than an individual peptide, bound to MHC mediates positive selection of a substantial number of thymocytes (7). The diverse set of self-peptides may also include peptides exclusively expressed by thymic epithelium or peptides predisposed to positively select T cells (8–11). Because the diversity of TCRs exceeds greatly the diversity of peptides bound to MHC molecules, it has also been postulated that peptide recognition during positive selection can be promiscuous, allowing a significant number of TCRs to be selected by a limited set of MHC-peptide ligands (12).

To examine the sensitivity of T cell selection with regard to peptide diversity, we tested whether an incremental increase in the number of peptides bound to class II MHC will have noticeable impact on the outcome of positive selection of CD4\(^+\) T cells in vivo. For that purpose, we have analyzed repertoires of TCRs found on CD4\(^+\) T cells in mice expressing MHC class II molecules (A\(^\alpha\)) covalently bound with two peptides. We have previously found that mice with covalently bound Ep\(_{52–68}\) Peptide (Ep) had a semidiverse repertoire of TCRs specific for various antigenic peptides (13, 14). Without the same molecular strategy, we have made transgenic mice that express A\(^\alpha\) molecules covalently bound with a close analogue of Ep peptide, in which the residue at position 58(G) was substituted with lysine (Ep58K). The altered amino acid was previously mapped as a TCR contact residue, presumably oriented toward the variable region of the TCR\(\alpha\)-chain, which lies over the N terminus of the peptide in the orthogonal orientation of TCR and MHC class I complexes (15, 16). We tethered another Ep-like peptide to the A\(^\alpha\) to minimize the overall conformational differences in A\(^\alpha\)Ep and A\(^\alpha\)Ep58K complexes, thereby favoring the same framework MHC-TCR contacts but with a single difference in the peptide-derived TCR contact residue. The new A\(^\alpha\)/Ep58K-transgenic mice were backcrossed with mice devoid of endogenous A\(^\beta\)-chain and invariant chain (I\(\iota\)). In the following experiments, we compared the diversity of TCRs expressing common transgenic TCR\(\beta\)-chain that are positively selected in vivo on thymic epithelium expressing exclusively A\(^\alpha\)Ep, A\(^\alpha\)Ep58K, or both A\(^\alpha\)Ep/A\(^\alpha\)Ep58K complexes. Spectratyping and sequence analysis of endogenously rearranged TCR\(\alpha\)-chains revealed that different sets of TCRs are selected in vivo by each of these MHC-peptide complexes. Additionally, we found that the TCR repertoire positively selected on both covalent A\(^\alpha\)-peptide complexes included a number of different TCRs. These results show that two class II MHC-peptide complexes expressed separately or together positively select a number of different TCRs.

Materials and Methods

Mice and chimeras

Mice expressing the transgenic A\(^\alpha\)Ep complex were generated at the National Jewish Medical and Research Center (Denver, CO) as previously described (13). The same cloning strategy was used to generate transgenic mice expressing the A\(^\alpha\)Ep58K complex. Both types of mice expressing covalent A\(^\alpha\)-peptide complexes were further backcrossed with mice deficient for the invariant chain (I\(\iota\)), endogenous A\(\beta\) chain (A\(^{\beta+}\)), and \(\beta\)-microglobulin (\(\beta\)-m) as indicated. Mice transgenic for the TCR V\(\beta\)14J\(\beta\)26 chain were generated in our laboratory (P. Kraj, data not shown) and were crossed once with mice that lack endogenous TCR\(\alpha\)-chain (TCR\(\beta^{\beta+}\alpha^{\alpha+}\)). Radiation chimeras were generated by irradiating 6- to 10-wk-old hosts with 1000 rad, followed by reconstitution with 3 \(\times\) 10\(^6\) T cell-depleted bone marrow from TCR\(\beta^{\beta+}\alpha^{\alpha+}\) mice. Mice deficient in

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2 Abbreviations used in this paper: wt, wild type; A\(^\alpha\), MHC class II molecule; Ep, Ep\(_{52–68}\) peptide; \(\beta\)-m, \(\beta\)-microglobulin; FTOC, fetal thymic organ culture; CDR3, complementarity-determining region.

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expression of endogenous TCR-α chain or β₂m were purchased from The Jackson Laboratory (Bar Harbor, ME).

**T cell hybridomas**

T cell hybridomas β₅m4.2 and IgM77.1, specific for β₅M₄₈·₅₈ and IgM₃₇₇·₃₉₂ peptides, respectively, were kindly provided to us by Dr. A. Y. Rudensky (Seattle, WA).

**Fetal thymic organ cultures (FTOC)**

Thymic lobes from 16-day gestational fetuses were placed onto nitrocellulose filters (Millipore, Bedford, MA) supported by a gelatin sponge (Geloflow; Pharmacia, Piscataway, NJ) in a 24-well plate and were incubated for 7–8 days at 37°C in DMEM high glucose medium (Cellgro, Herndon, VA) supplemented with 10% FBS (Life Technologies, Rockville, MD), 2 mM L-glutamine, 50 μg/mL nonessential amino acids, penicillin, and streptomycin. The Abs and media were replenished every day until indicated.

**Isolation and purification of CD₄⁺ Vβ14⁺-transgenic T cells**

Single-cell suspensions from the pooled axillary, inguinal, mesenteric, and paraaortic lymph nodes were incubated with MACS MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) conjugated to anti-CD8 for 15 min at 4°C, washed, and sorted using an autoMACS cell separator (Miltenyi Biotec). The negative fraction, depleted of CD₈⁺ T cells, was incubated with anti-Vβ14-biotin (PharMingen, San Diego, CA) for 15 min at 4°C and was washed and incubated again with MACS MicroBeads conjugated to streptavidin for 15 min at 4°C. The cells were then washed and sorted using an autoMACS cell separator. The positive fraction contained CD₄⁺ Vβ14⁺ T cells of >97% purity.

**RNA extraction and cDNA synthesis**

Total RNA was extracted from 2 × 10⁶ CD₄⁺ Vβ14⁺ T cells using UltraTaq RNA reagent (Biotec, Laboratories, Houston, TX) and was converted to cDNA using the reverse transcription system (Promega, Madison, WI).

**Spectratyping analysis of Va1-Va4 repertoires**

The technique used in this study was described elsewhere (14). In brief, cDNA was amplified in a standard PCR (35 cycles) with Va1(1–4)-specific sense primers and an antisense primer complementary to the Ca region (17) using 1/50 of the cDNA previously generated per reaction. One to 5 μL of PCR product was used as a template for a runoff reaction with a standard PCR with Vα14 (1–4)-specific sense primers and an antisense primer complementary to the Cα region. The denatured runoff products were separated on a 6% polyacrylamide gel, and the bands were analyzed with an ABI Prism 377 DNA sequencer (PE Applied Biosystems, Foster City, CA) using GeneScan software. The bands are expressed in graphic form as a spectrum with peaks, with the area of each peak corresponding to the intensity of the band. The relative intensity of the band was calculated by comparing it to the combined intensity in the particular VJ rearrangement.

**Sequencing of Va3-Jα rearrangements with a given length**

This approach was described elsewhere (14). In brief, after 35 cycles of standard PCR with Va1(1–4)-specific sense primers and an antisense primer complementary to the Ca region, bands corresponding to the complementarity-determining region 3 (CDR3) of 9 and 10 aa in length and were excised from a 6% polyacrylamide gel after ethidium bromide staining. DNA was extracted from each band and was amplified by PCR and cloned into the pCR2.1 vector with the TOPO-TA cloning system (Invitrogen, San Diego, CA). Twenty randomly picked colonies were sequenced with Va3 primer using the ABI Prism 377 DNA sequencer. The CDR3 sequences were veriﬁed to cDNA using the reverse transcription system (Promega, Madison, WI).

**Cell preparations and flow cytometry analysis**

Single-cell suspensions were prepared from thymi and spleen by mechanical disruption. Spleen cell suspensions were additionally incubated with buffered ammonium chloride to remove RBC. To analyze stromal epithelial cells in suspension, thymi were incubated for 30 min at 37°C with Collagenase (1 mg/ml, type IV; Sigma, St. Louis, MO) and DNase (0.02 mg/ml, bovine pancreatic DNase I; Sigma) followed by incubation with EDTA (0.1 M) for 5 min, then ﬁltered to remove debris and used for FACS analysis. The following Abs were used for flow cytometry analysis: anti-Vβ14-FITC, anti-C4D-PE, anti-C4D-APC, anti-Cd8-PerCP, BP-1-PE (all from PharMingen), anti-TCR-FITC, Y3P-FTTC, and B220-biotin (pre-pared in our laboratory). Biotinylated Ab was detected with streptavidi-nin-PE (PharMingen). Staining was done on ice in 1× wash buffer (balanced salt solution containing 2% FBS and 0.1% NaN₃). All FACS analysis was performed using FACS Calibur flow cytometer (Becton Dickinson, Mountain View, CA) and CellQuest software (Becton Dickinson). Dead cells were excluded by gating of forward and side scatter.

**Results**

The covalent AβEp and AβEp58K complexes contribute to the positive selection of the majority of CD4⁺ T cells in AβEpli and AβEp58Kli mice

To determine the role of peptide in positive selection of CD4⁺ T cells, we produced two lines of transgenic mice that expressed Aβ molecules covalently bound with two peptides. Expression of Aβ on peripheral APCs was similar in both lines of transgenic mice but was reduced >10-fold in comparison to wt mice. However, expression of Aβ on thymic epithelial cells was similar in the transgenic and wt mice (Fig. 1, A and B). The new covalent AβEp58K complex was detected by the YAE mAb, which was originally described as specific for the AβEp complex (18, 19). The AβEp58K complex was also recognized by some T cell hybridomas specific for the AβEp complex (data not shown). Staining of the AβEp58K complex by the peptide-independent Y3P mAb was abolished in the presence of the YAE mAb (data not shown), implying that the detectable Aβ molecules remain covalently bound with Ep58K peptide. Moreover, splenic APCs expressing the AβEp58K complex did not present a detectable amount of endogenous peptides to two T cell hybridomas specific for β₅M₄₈·₅₈ or IgM₃₇₇·₃₉₂ peptide (20) (Fig. 1, C and D). These results showed that both covalent...
Aβ-peptide complexes have common conformational domains that are recognized by Abs and specific T cells and that both peptides are firmly attached to Aβ.

To prove that the covalent Aβ-peptide complexes select CD4+ T cells in AβEpIi− or AβEp58Kli− mice, we cultured 16-day-old fetal thymus from each transgenic line in the presence of YAe or Y3P mAbs. Although complete inhibition of the differentiation of CD4+ thymocytes using mAb in FTOCs is difficult to obtain (21), both YAe and Y3P mAbs comparably blocked the development of CD4+ thymocytes in FTOCs (Fig. 2). Blocking with YAe mAb was better in FTOCs derived from AβEpIi− fetuses. This was most likely due to the reduced affinity of this Ab for the AβEp58K complex (an exchanged residue is included in the epitope recognized by YAe) and the significantly higher expression of the Ab Ep58K peptide complex on bone marrow-derived thymic stromal cells (Fig. 1A). The number of CD4+ thymocytes was around 50% or less in FTOCs incubated with YAe mAb and was better in FTOCs derived from AβEpIi− fetuses. This was most likely due to the reduced affinity of this Ab for the AβEp58K complex (an exchanged residue is included in the epitope recognized by YAe) and the significantly higher expression of the Ab Ep58K complex on bone marrow-derived thymic stromal cells (Fig. 1A).

The number of CD4+ thymocytes was around 50% or less in FTOCs incubated with YAe mAb, indicating that the covalent Aβ-peptide complexes selected a substantial population of CD4+ T cells. Collectively, the results obtained from in vitro peptide presentation and FTOC experiments suggested that the covalent peptides remain bound to Aβ and significantly, if not exclusively, select CD4+ T cells.

Covalent Aβ-peptide complexes positively select a number of CD4+ T cells expressing a transgenic TCRβ-chain and endogenously rearranged TCRα-chains

An initial analysis of the TCR Vβ segment distribution in AβEpIi− or AβEp58Kli− mice revealed that, despite the limited number of CD4+ T cells, both repertoires consist of many different TCRs (data not shown). Furthermore, in mice expressing each of the covalent Aβ-peptide complexes, positive and negative selection of T cells was mediated by different peptide species, so an imprint of the particular peptide on positive selection was difficult to investigate. Hence, we lethally irradiated AβEpIi−, AβEp58Kli−, and double peptide AβEpAβEp58Kli− mice and separately reconstituted them with bone marrow from mice transgenic for TCRβ14−, which had only a single functional TCRα locus (TCRA+/−) and expressed Aβ molecules loaded with wt peptides (Aβwt). Expression of the transgenic TCRβ14− chain forced all T cells to express the same TCRβ-chain, while the preservation of only one functional, endogenous TCRα locus ensured that the expressed TCRα-chain was used in thymic selection. Therefore, in radiation chimeras, thymocytes will survive only if their TCRs, composed of a single transgenic TCRβ-chain coupled to the rearranged endogenous TCRα-chain, weakly bind covalent Aβ-peptide complex(es). To minimize restraints that might occur from the arbitrarily mismatched transgenic TCRβ-chain and covalent Aβ-peptide complex, we used transgenic TCRβ-chain derived from the TCR originally positively selected in vivo on the AβEp complex (23). Furthermore, reconstitution of the AβEpIi− or AβEp58Kli− mice with bone marrow cells bearing Aβwt normalized negative selection to the same set of endogenously derived peptides and emphasized the role of the two covalent peptides bound to Aβ on the positive selection of CD4+ T cells. Finally, the possibility that one of the covalently attached peptides could negatively select some of the TCRs was controlled by the use of double Aβ-peptide chimeras, where both covalent peptides were simultaneously expressed.

In AβEp58Kli− and AβEpIi− chimeras reconstituted with the Aβwt TCRβ14− bone marrow, a few Vβ14+CD4+ T cells were positively selected (Fig. 3). Roughly three times more transgenic CD4+ thymocytes was found positively selected by the AβEp58K complex than by the AβEp complex, implying that 58K residue may be important for low-affinity interactions between the covalent MHC-peptide complex and multiple αβTCRs. Staining with mAbs specific for different Vα segments indicated multiple

FIGURE 2. The Ep and Ep58K peptides significantly contribute to positive selection of CD4+ T cells in single peptide mice. FTOCs from (A) AβEp58K or (B) AβEp mice cultured in the presence of the indicated Abs are shown as CD4 vs CD8 plots of TCR intermediate and high gated thymocytes. One lobe from each day 16 fetal thymus was cultured in medium only (control) and the other lobe was cultured with either Y3P or YAe at the final concentration of 40 μg/ml. For each set of conditions, three thymi were analyzed. A representative experiment is shown. In a control experiment, YAe Ab did not block positive selection of CD4+ T cells in FTOCs from Aβwt mice (data not shown).
rearrangements of endogenous TCRα-chains (data not shown), proving that the transgenic Vβ14+ chain associates with different endogenous TCRα-chains.

**Different repertoires of TCRs are selected in mice expressing similar Aβ-peptide complexes**

To further assess the diversity of TCRα-chains selected in vivo by AβEp or AβEp58K complexes, we used a high-resolution RT-PCR method that visualizes the spectrum of sizes of the TCRα CDR3 (14). We amplified rearrangements of four randomly picked TCRα families (Vα1-Vα4). The size distribution of PCR products was visualized as bands of different intensity after separation on the sequencing gel. To exclude the possibility that different peaks reflect random PCR amplification, the reproducibility of the profiles was verified in four separate PCRs, which yielded identical results (Fig. 4A). The analysis of the CDR3 of four related Vα-chains associated on CD4+ T cells with transgenic Vβ14+ chain revealed significant differences between the TCRs positively selected by the AβEp or AβEp58K complex. For example, the pattern of Vα1 selected on the AβEp58K complex was bell shaped, indicating scattered rearrangements, while the same Vα1 family selected on AβEp complex was dominated by CD4 single-positive thymocytes. Data are representative of analyses on at least three chimeras of each group.

![FIGURE 3. Selection of TCRβTgα1/2 CD4+ T cells on thymic epithelium expressing AβEp or AβEp58K complexes. The left column shows CD4 vs CD8 plots, the right column shows Vβ14 expression on gated CD4 single-positive thymocytes. Data are representative of analyses on at least three chimeras of each group.](http://www.jimmunol.org/)

![FIGURE 4. Strong influence of peptide on profiles of analyzed CDR3s of the TCRα-chains positively selected in single or double peptide mice. The peaks corresponding to CDR3 with a length of 10 aa are indicated with a vertical dotted line. A, Results of two representative experiments displaying CDR3 polymorphism in the four analyzed TCR Vα families are shown. B, Similar profiles of the TCRα-chains positively selected on AβEp58KIi complex in the absence or presence of βm-dependent, non-classical molecules. All profiles shown are representative of at least four independent experiments.](http://www.jimmunol.org/)
$\text{A}^b\text{Ep58Kli}^{-}$ mice expressing or devoid of nonclassical class I MHC molecules, indicating that the observed diversity of the TCR-$\alpha$-chains is derived from recognition of the peptides bound to A$^b$.

To determine the degree of diversity of endogenous TCR-$\alpha$-chains selected by A$^b$Ep, A$^b$Ep58K, or A$^b$EpA$^b$Ep58K complexes, we excised gel fragments containing amplified CDR3 sequences that were 9–10 aa long and were derived from V$\alpha3^{+}$ TCRs. The CDR3 sequences of this length were commonly represented in the V$\alpha$-$\text{chains}$ selected by A$^b$Ep, A$^b$Ep58K, or A$^b$EpA$^b$Ep58K complexes, but were relatively underrepresented in the TCR$\alpha3^{+}$ profile amplified from mice expressing the A$^b$Ep$\text{complex}$. Fifty-six of these were sequenced. As shown in Table I, 39 CDR3 sequences differed at the protein level and 44 at the nucleotide level (data not shown), implying that TCR$\alpha$-$\text{chains}$ rearranged to the dominant J$\alpha$ segments (J$\alpha3$) on the nucleotide level (data not shown), implying that TCR$\alpha$-$\text{chains}$ repertoires are diverse in all mice. In both single, but not double, A$^b$-peptide chimeras, >30% of the V$\alpha3^{+}$ segments were found rearranged to the dominant J$\alpha24$ segment. Additionally, 90% of the V$\alpha3^{+}$ TCRs selected by A$^b$Ep complex used one of three J$\alpha$ segments (J$\alpha1$, J$\alpha24$, and J$\alpha40$), whereas V$\alpha3^{+}$ TCRs selected in chimeras expressing A$^b$Ep58K or A$^b$EpA$^b$Ep58K complexes used 9 or 12 different J$\alpha$ regions, respectively. The lower number of the repetitive CDR3 sequences and the greater number of J$\alpha$ segments found in “double peptide” mice suggested that the diversity of the TCR repertoire may be increased due to an additive effect of selection on both A$^b$-peptide ligands (also visible in the CDR3 profiles of V$\alpha1^{+}$, V$\alpha2^{+}$, and V$\alpha4^{+}$ TCR). Four CDR3 sequences (10, 17, 19, 21) were found in more than one type of chimera, and one CDR3 (17) was present in all three chimeras. These CDR3s may represent TCRs selected on A$^b$ with little peptide contribution. The CDR3s of clones 17, 19, 20, and 37 were identical at the protein level but different at the nucleotide level. These clones probably represent TCRs subject to selective pressure for a particular CDR3 amino acid sequence (24).

**Discussion**

Recent studies using mice with MHC molecules bound with one peptide, or a dominant peptide, have provided important information on the role of peptides in thymic selection, peripheral survival, and allore cognition (24–28). We compared repertoires of TCRs positively selected in vivo on two covalent peptides bound to A$^b$ via the same anchor residues, to determine whether these TCRs

### Table I. Sequences of V$\alpha$-J$\alpha$ rearrangements contributing to a CDR3 length of 9 and 10 aa

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<td>KGRGTSKLS</td>
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| Ratio of CDR3 9 aa/10 aa | 9/10 | 0/18 | 12/7 |

$^{\alpha}$ The CDR3 includes amino acids beginning with the third amino acid after the invariant C residue in all TCRAV genes (Y-L/F-C-A-X-1) and spans to the amino acid immediately preceding the TCRAJ motif (2-F-G-X-F-G-T).
repetitories would be similar or different. Furthermore, to investigate whether the diversity of αβ TCRs would change due to incremental increase in the number of selecting MHC-peptide ligands, we characterized the repertoire of TCRs positively selected on thymic epithelium that coexpressed both covalent Aβ-peptide complexes. All analyzed TCRs expressed one TCRβ-chain, were tolerant to wt peptides bound to Aβ, and used different endogenously rearranged TCRα-chains.

First, we found that two covalent Aβ-peptide complexes select different number of CD4+ T cells in vivo. This finding is not surprising in the context of the earlier report where different individual peptides added to FTOC positively selected various number of thymocytes (29). The number of CD4+ T cells (both TCR transgenic or nontransgenic) selected by AαEp58K complex exceeded the number of CD4+ T cells selected on Aαwt in the absence of li (R. Pacholczyk, unpublished observations). Moreover, two other transgenic mouse lines expressing Aβ covalently bound with Ep analogue with three residues (58, 61, and 63) substituted with lysine or exogenous peptide pigeon cytochrome c 43–58 had the same number of selected CD4+ T cells as mice expressing AαEp complex (R. Pacholczyk, manuscript in preparation). These two former peptides bind Aβ with low affinity and could be efficiently replaced with exogenously added peptides, implying that more efficient selection of CD4+ T cells in AαEp58K li− mice is not simply a result of a “leak” of endogenously derived peptides (also see below). Conceivably, a higher level of expression of the AαEp58K complex might result in increased positive selection of CD4+ T cells. However, this interpretation is at odds with the outcome of positive selection in transgenic mice expressing various amounts of the AαEp complexes (30). In these studies the transgenic animals expressing the highest level of the AαEp complex had the lowest number of selected CD4+ T cells.

It is also possible that replacement of the glycine in position 58 by lysine might reduce overall the affinity of interactions between the covalent Aβ-peptide complex and multiple, endogenous TCRα-chains. Therefore results of our experiments do not support the hypothesis that class II MHC-peptide complexes, with the flat surface exposed toward the TCR, are predisposed to positively select the highest number of CD4+ T cells (31). Instead, one may imagine that the large side chain projecting toward the TCRα-chain may bind more TCRs with low affinity, which is sufficient to induce positive selection of considerably more CD4+ T cells.

Second, we found that TCR repertoire selected on each of the Aβ-peptide complexes are different. Although our experimental setup with fixed TCRβ-chain and TCRα-chain positioned over the substituted residue in the covalently bound peptides might enhance the TCRα-chain diversity, the lack of more significant overlap between the two repertoires selected on each of the single Aβ-peptide complexes was unexpected. The differences between the selected TCR repertoires were noticed either by TCRα CDR3 spectratyping or DNA sequencing of the limited sets of the TCRα-chains. Of 39 different CDR3 sequences cloned from two “single peptide” mice, only one CDR3 sequence was the same at the amino acid level.

Finally, we found that coexpression of both covalent Aβ-peptide complexes results in selection of TCRs that are different from the ones found in mice expressing individual Aβ-peptide complexes. For example, the Vα4+ CDR3 profiles selected on AαEpAαEp58K complexes had the highest number of different lengths. These findings implied that the individual Aβ-peptide complex has an additive effect on the selected TCR repertoire. The sequencing of a narrow set of Vα3+ CDR3s that are only 9–10 aa long and were selected on tested Aβ-peptide complexes revealed that these sequences are different between the analyzed repertoires. It is also possible that the different TCRs found in these mice appeared as a result of the changed density of each covalent Aβ-peptide complex. Alternatively, these TCRs could use a “gemish” of both covalent Aβ-peptide complexes (7). Because more CD4+ T cells were found in mice expressing the AαEp58K, rather than the AαEp complex, conceivably, the former Aβ-peptide complex likely contributed more to positive selection in “double peptide” mice. One may doubt that the Vα3+CDR3 sequences presented here do not sufficiently represent TCRs selected on the covalent Aβ-peptide complexes. Although we may have not sequenced all relevant CDR3s, the analyzed TCRα-chain sequences were restrained not only by pairing with a fixed TCRβ-chain and by positive selection on covalent Aβ-peptide complexes, but also by extensive negative selection and the narrowed length of CDR3s, which was not executed in the previously published studies (24).

One may interpret that the observed difference in selected TCRs in AαEpi− vs AαEp58Kli− mice comes only from different contributions of low-abundance peptides (21). The following argues against this interpretation. The blocking experiments in FTOCs showed that at least the vast majority of CD4+ T cells is selected on covalently bound peptides. The number of the CD4+ T cells in double peptide mice is not a simple sum of the number seen in single peptide mice, regardless of the “doubled” vulnerability for leakage of endogenous peptides. Additionally, sequencing of the relatively small number of CDR3 sequences reveals selection at the protein level, which we would not see if these TCRs were selected on many low-abundant MHC-peptide complexes (21).

In retrospect, similar studies have examined TCRs on CD4+ thymocytes selected in wt or H-2M-deficient mice, where the latter expressed Aα molecules predominantly bound with li-derived class II-associated li peptide (24). The TCRs selected in H-2M-deficient mice had limited sequence diversity in the CDR3s of a particular TCRα-chain, prompting the conclusion that positive selection primarily shapes the repertoire of TCRs expressed on peripheral T cells. However, the spectra of self-peptides bound to class II MHC in wt and H-2M− mice differ significantly (32, 33) and therefore those experiments could not assess the contribution of the individual peptide to the positive selection of αβ TCR expressed on CD4+ T cells. In this study, by comparing αβ TCRs positively selected on thymic epithelium expressing two covalent Aβ-peptide complexes we show that each of these selects a number of different TCRs. Thymic cortical epithelium express 10-fold less class II MHC molecules than peripheral APCs, and are likely to be bound by 103–104 endogenous peptides (9, 34). The lowest limit of different αβ TCRs expressed on naive T cells in blood has been estimated at 2.5 × 107, implicating that an impact of the individual MHC-peptide complex on positive selection of T cells may indeed be detectable (14, 35). Consequently, we found that as the diversity of positively selecting Aβ-peptide complexes increases incrementally, the repertoire of TCRs expressed on CD4+ T cells changes, demonstrating how a restrained set of self-MHC-peptide complexes manages to select a large repertoire of αβ TCRs.

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References


