Quantitative Analysis of the T Cell Repertoire Selected by a Single Peptide–Major Histocompatibility Complex

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Summary

The positive selection of CD4$^+$ T cells requires the expression of major histocompatibility complex (MHC) class II molecules in the thymus, but the role of self-peptides complexed to class II molecules is still a matter of debate. Recently, it was observed that transgenic mice expressing a single peptide–MHC class II complex positively select significant numbers of diverse CD4$^+$ T cells in the thymus. However, the number of selected T cell specificities has not been evaluated so far. Here, we have sequenced 700 junctional complementarity determining regions 3 (CDR3) from T cell receptors (TCRs) carrying Vβ11-Jβ1.1 or Vβ12-Jβ1.1 rearrangements. We found that a single peptide–MHC class II complex positively selects at least 10^5 different Vβ rearrangements. Our data yield a first evaluation of the size of the T cell repertoire. In addition, they provide evidence that the single Eα52-68–I-A^b complex skews the amino acid frequency in the TCR CDR3 loop of positively selected T cells. A detailed analysis of CDR3 sequences indicates that a fraction of the β chain repertoire bears the imprint of the selecting self-peptide.

Key words: thymus • major histocompatibility complex • T cell receptors • repertoire development • transgenic/knockout

During development, thymocytes undergo two steps of selection, each involving interaction with self-MHC molecules (1–4). Positive selection rescues thymocytes from programmed cell death and ensures that the mature T cell repertoire is directed against foreign peptides bound to self-MHC molecules (5–8). Subsequently, negative selection eliminates, through clonal deletion, T cells with potentially autoreactive receptors (1, 2, 9, 10).

Using in vitro fetal thymic organ culture system from β2 microglobulin- or transporters associated with antigen processing (TAP)-deficient mice, it was shown that peptides were important for positive selection of CD8$^+$ T cells (11, 12). Similar experiments with mice expressing rearranged TCR genes of known specificity revealed a stringent requirement for peptide recognition during the positive selection process (13–16). Affinity measurements of TCR-peptide–MHC interaction indicated that peptide–MHC complexes capable of positive selection were of low affinity for the TCR, whereas high affinity ones were deleting ligands (17). More recently, natural self-peptides, extracted from MHC class I groove and capable of driving positive selection, were identified (18, 19). It was shown that different peptides could select thymocytes expressing different TCRs, suggesting that weak but specific interactions with self-peptide–MHC complexes promote positive selection of CD8$^+$ T cells. Hence, a particular TCR could be selected by different peptides.

Peptide involvement in positive selection of CD4$^+$ T cells was addressed using genetically engineered mice designed to express MHC class II molecules complexed to a single peptide. In mice lacking the MHC-encoded H-2M molecule and involved in the removal of the class II–associated invariant chain peptide (CLIP)[1] during the MHC class II maturation process (20–23), almost all MHC class II

[1]*Abbreviations used in this paper:* CLIP, class II–associated invariant chain peptide; Tg, transgenic mice.

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molecules are occupied with the CLIP peptide (24). Transgenic mice (Tg) for the I-Aβ chain connected to the Eα52-68 peptide (25, 26) backcrossed to MHC class II– and invariant chain–deficient animals express the single Eα52-68 peptide–I-A^b complex. Studies conducted with these two types of Tg suggested that a large and diverse repertoire of $CD4^+$ T cells is selected (22–27). Staining with anti-Vβ and -Vα antibodies showed that a close to normal spectrum of Vβ and Vα was used by mature $CD4^+$ T cells. Sequencing studies in the H-$2M^{-}/^{-}$ model, from two Vβs and one Vα, confirmed the polyclonality (24). Finally, positively selected cells were capable of responding to immunization with several peptide antigens (22–24, 27). In the Eα52-68–I-A^b model, Fukui et al. (26) have shown that the level of expression of this complex in the thymus affects the $CD4^+$ T cell selection dramatically. Transgenic lines with low expression of Eα52-68–I-A^b complexes positively select $CD4^+$ T cells, whereas such cells are eliminated in the thymus of another line with high expression (26). Furthermore, two thirds of the selected $CD4^+$ T cells react with the syngeneic cells that express the same MHC class II molecules complexed to the natural set of self-peptides (25). In wild-type mice, such lymphocytes are eliminated by negative selection on bone marrow–derived cells expressing wild-type class II molecules in the thymus (23, 24). These results indicate that the Tg repertoire of $CD4^+$ T cells is different from the wild type. Furthermore, it was shown using mice expressing various transgenic TCRs (which are positively selected in mice expressing wild-type class II molecules) that these TCRs are not selected in the CLIP mice or the Eα52-68–I-A^b Tg (22–24). These observations imply that the selecting peptide influences, to some extent, the emerging repertoire. However, the diversity of the repertoire selected by a single peptide–MHC class II complex has not been quantitated so far. In addition, the available evidence does not rule out that a few positively selected specific TCR rearrangements have not yet been detected over a polyclonal background.

These studies were designed to quantitate the number of positively selected T cells. We have analyzed the α and β T cell repertoire of $CD4^+$ T lymphocytes selected by the Eα52-68–I-A^b complex extensively. We show that $CD4^+$ $CD8^-$$NK1.1^-$$HSA^-$ thymocytes selected on this complex bear TCRs that include all Vβs and 10 Vαs tested. The Jβ usage and CDR3 length distribution of Vβ and Vα chain rearrangements are indistinguishable from the repertoire of $CD4^+$$CD8^-$$NK1.1^-$$HSA^-$ thymocytes from normal C57Bl/6 mice. Extensive sequencing of particular Vβ–Jβ combinations with the same CDR3 length enabled us to calculate that a minimum of 10^5 different Vβ rearrangements are selected by the single peptide–MHC class II complex. Careful analysis of these sequences revealed some differences in the CDR3 amino acid composition of T cells selected by the single peptide–MHC complex or by wild-type MHC class II molecules. Altogether, our results provide a lower limit on the size of the selected $CD4^+$ T cell repertoire in vivo and indicate that part of the repertoire bears the imprint of the selecting peptide.

Materials and Methods

Animals. Mice used in this study have been described elsewhere (26) and were bred in the animal facility at the Medical Institute of Bioregulation. In brief, Tg were produced by injection of DNA encoding the β chain of I-A^b covalently linked to the peptide derived from MHC class II Eα (Eα52-68) into fertilized eggs of I-$A\beta^b$ gene knockout (I-$A^{-/-}$) mice carrying H-2^b haplotype (28). To avoid peptide replacement, Tg were backcrossed with mice deficient for the invariant chain ($Ii^{-/-}$; reference 29). The B2L mice that express ∼10% of the level of I-A^b found in C57Bl/6 animals were chosen for all studies.

Purification of $CD4^+$ T Cells. Thymocytes were prepared from 6-wk-old mice and counted. $CD4^+$$CD8^-$$NK1.1^-$$HSA^-$ T cells were prepared using depletion and cell sorting as previously described (26). In brief, thymocytes were incubated with anti-CD8 and anti-HSA (J11D) monoclonal antibodies and killed by addition of rabbit complement. Remaining living cells were stained with anti-CD4-PE, anti-CD8-FITC, and anti-NK1.1-FITC (PharMingen, San Diego, CA). $CD4^+$$CD8^-$$NK1.1^-$$HSA^-$ T cells were analyzed and sorted on an Epics cell sorter (Coulter Corp., Miami, FL). For Immunoscope analysis of the T cell receptor repertoire, cells were washed with PBS and immediately frozen down into liquid nitrogen until further manipulation.

mRNA Extraction and cDNA Synthesis. $Poly(A)^+$ mRNA from $CD4^+$$CD8^-$$NK1.1^-$$HSA^-$ thymocytes was extracted (Quick mRNA Micro Prep Kit; Pharmacia, Piscataway, NJ). In brief, cells were lysed in guanidium thiocyanate and mRNA samples were purified by affinity chromatography on oligo-d(T) cellulose. After ethanol precipitation, mRNAs were reverse transcribed into cDNA using a cDNA synthesis kit (Boehringer Mannheim GmbH, Mannheim, Germany). RNA were denaturated at 70°C for 10 min and then incubated with random primers (5 μM), dNTP (1 mM), Rnasin (40 U; Promega, Madison, WI) and 2 U of reverse transcriptase from avian myeloblastosis virus (Boehringer Mannheim GmbH) at 43°C for 1 h, followed by an incubation at 53°C for 10 min.

Immunoscope Analysis of Vβ and Vα Repertoires. PCR was carried out in 50 μl on 1/50 of the cDNA with 2 U of Taq polymerase (Goldstar, Eurogentec, Seraing, Belgium) in the buffer provided by the supplier. Each Vβ mRNA was amplified using one of a set of 23 Vβ-specific sense primers and an antisense primer designed to hybridize in the Cβ gene (30). Similarly, Vα mRNAs coding for Vα 1, 2, 3, 4, 5, 6, 8, 10, 11, 17, 18, and 19 were amplified with Vα-specific sense primers and an antisense primer from the Cα region (31).

Each amplified product was then used as a template for an elongation reaction with oligonucleotides labeled with a fluorescent tag (runoff reactions). The fluorescent runoff products, corresponding to the elongation of individual Vβ or Vα PCR products with various CDR3 sizes, were loaded on polyacrylamide gels and subjected to electrophoresis in an automated DNA sequencer. CDR3 size distribution and signal intensities were then analyzed with the Immunoscope software (30, 32, 33). The patterns observed from unprimed lymph node cells or splenocytes usually contain six to eight size peaks each spaced by three nucleotides, corresponding to the lengths of in-frame transcripts. The area of each size peak is proportional to the quantity of the TCR transcripts of the corresponding CDR3 length in the sample. It should be noted that each peak corresponding to a given CDR3 length is likely to contain multiple distinct sequences. Increase in the height and area of a size peak signals clonal expansion, occurring against polyclonal background. The fluorescent Cβ (or Cα) primers used in the runoff reactions reveal all Vβ-Jβ (Vα-Jα) rearrangements. Runoff reactions using specific fluorescent Jβ primers reveal the CDR3 length distributions from particular V-J combinations. In brief, 2 μl of each PCR product was subjected to three to five cycles of elongation with dye labeled specific primer (0.1 μM) in a final volume of 10 μl, containing 0.2 mM dNTP, 3 mM $MgCl_2$, and 0.2 U of Taq polymerase (Promega) in buffer (Promega). The elongation starts with 1 min at 94°C, followed by three to five cycles each consisting of 45 s at 94°C, 45 s at 60°C, 45 s at 72°C, and ending with a step at 72°C for 3 min. Each runoff product was then diluted vol/vol in 30 mM EDTA-formamide solution. This mix was heat-denatured during 10 min at 80°C and 2 μl aliquot was loaded on a 6% (or 4.25%) polyacrylamide 8 M urea gel. Gel electrophoreses were performed on a DNA sequencer (373A or 377; PE Applied Biosystems, Foster City, CA). Peak size and fluorescent intensity were determined with the Immunoscope software (32, 34–36). Because the technique is semiquantitative, we could evaluate the relative frequency of Jβ usage for a given Vβ population, as described in reference 37. The ratio of the area of the peak generated with a given Jβ primer to the areas of all the Jβ primers was calculated as a measure of the relative frequency of Jβ usage in the $V\beta^+$ cell population under study.

Sequencing of Particular Vβ-Jβ Rearrangements with a Given Length. Two PCR reactions were performed using specific Vβ11-Jβ1.1 or Vβ12-Jβ1.1 primers in 50 μl on 1/50 of the cDNA with 2 U of Taq polymerase (Goldstar, Eurogentec) in the supplier's buffer. The elongation starts with 1 min at 94°C, followed by 40 cycles each consisting of 45 s at 94°C, 45 s at 60°C, 45 s at 72°C, and ending with a step at 72°C for 3 min. The PCR product was ethanol precipitated and resuspended in 10 μl formamide containing 0.05% bromophenol blue and 0.05% xylene cyanol. This mix was heat denatured for 10 min at 80°C and then loaded on a 8% polyacrylamide/7 M urea gel. After migration, PCR products were visualized by a silver coloration (DNA Silver Staining System; Promega) following manufacturer's instructions. We usually obtained six to eight bands each spaced by three nucleotides, corresponding to in-frame transcripts of the V-J combinations. Bands of interest, corresponding to a given CDR3 length, were cut from the gel and disrupted in 40 μl of water. A second PCR was carried out using the same primers on 2 μl of the isolated PCR product with 2 U of Taq polymerase (Goldstar, Eurogentec) in the supplier buffer for 20 cycles. Further purification was realized on a 15% nondenaturating acrylamide gel in TBE. Staining of this gel was obtained with a 30 min bath in a 0.5 mg/ml ethidium bromide solution in TBE (Tris-borate-EDTA). PCR product was electroeluted in TBE and purity of the sample was estimated by a runoff reaction with the fluorescent Jβ primer. We usually obtained 90–95% purity of the final product with the expected size. PCR products were then cloned in pCR®2.1 vector from the TA cloning kit (Invitrogen, Carlsbad, CA). 10–20 ng of DNA were ligated with an equimolar concentration of the vector. The ligation was carried out in 10 μl with 4 U of T4 DNA ligase during 12 h at 14°C, following the manufacturer's instructions. Competent INVαF′ bacteria (Invitrogen) were then transformed by heat shock at 42°C for 30 s and plated on Luria-Bertani medium containing 50 μg/ml ampicillin and 60 μg/ml X-Gal (ICN Biomedicals Inc., Aurora, OH).

For the purpose of sequencing, PCR was carried out directly on $LacZ^-$ colonies in a final volume of 30 μl using universal primers RP and M13(-40) designed to hybridize on each side of the polylinker where the Vβ-Jβ PCR product was cloned. 5 μl of this PCR product was treated during 40 min at 37°C with 1 U of shrimp alkaline phosphatase (Nycomed Amersham, Buckinghamshire, UK) and 10 U of exonuclease (Nycomed Amersham) in a final volume of 10 μl. Both enzymes were heat denatured at 80°C for 20 min. Sequencing reactions were carried out directly on these products using M13(-20) primer and the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit following the manufacturer's instructions (PE Applied Biosystems). Reaction products were loaded on a 4.25% polyacrylamide 8 M urea gel. Gel electrophoreses were performed on a DNA sequencer (377; PE Applied Biosystems), and CDR3 region corresponding sequences were extracted and analyzed using software designed for this purpose (Casrouge, A., E. Beaudoing, J.P. Levraud, L. Gapin, N. Garguier, D. Gautheret, J.M. Claverie, J. Kanellopoulos, P. Kourilisky, manuscript in preparation).

Results

CDR3 Size Distribution of TCR-α/β Repertoire from $CD4^+CD8^-NK1.1^-HSA^-$ Thymocytes Positively Selected by a Single Peptide–MHC Complex. Single positive CD4 thymocytes were purified from the thymus of B2L, which are transgenic for a single peptide–MHC class II complex (26). The repertoire analysis did not include $CD4^+CD8^-$ $NK1.1^+$ cells, which were eliminated by specific lysis with antibodies and complement, because it is known that such cells are selected by nonclassical MHC class I molecules such as CD1 (38).

mRNA from 3–4 × 10^5 $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes was extracted and reverse transcribed into cDNA. 23 Vβ- and 12 Vα-specific PCR reactions were then carried out. The product of each PCR was visualized by performing a runoff extension with internal Cβ- (or Cα-) specific fluorescent primer. Such primers enable elongation through the CDR3 region of each amplified product, and therefore reveal peaks of different sizes for all V-J combinations after separation on a sequencing gel. Previous studies from our laboratory, using unstimulated mouse splenocytes, have shown that, in all functional rearranged Vβ or Vα segments, the CDR3 size patterns display six to eight peaks spaced by three nucleotides. These peaks are distributed in Gaussian-like patterns that are characteristic of polyclonal T lymphocytes (34). The repertoire analysis performed on single positive CD4 cells selected on the Eα52-68 peptide–I-A^b complexes is shown in Fig. 1. 22 out of the 23 Vβ genes were amplified (Fig. 1 *A*). Amplification of Vβ6 gene failed for technical reasons. All Vα genes tested were amplified (Fig. 1 *B*). The size distribution of CDR3 from each Vα or Vβ family gives a set of about seven peaks, each spaced by three nucleotides and corresponding to in-frame transcripts. For Vβ17 and Vβ19 genes that are pseudogenes in C57Bl/6 mice (39–41), in-frame transcripts are difficult to detect above the background of out-of-frame transcripts. To detect more subtle modifications of the Vβ repertoire, we performed runoff reactions with 12 fluorescent primers that recognize individual Jβ segments for the Vβ4, Vβ8.2, Vβ10, Vβ11, Vβ12, and Vβ14 families. The CDR3 distribution of specific Vβ-Jβ rearrangements was then obtained for the six different Vβs of two individual Tg. Our results clearly show that for each Vβ, all Jβs are used. Furthermore, the

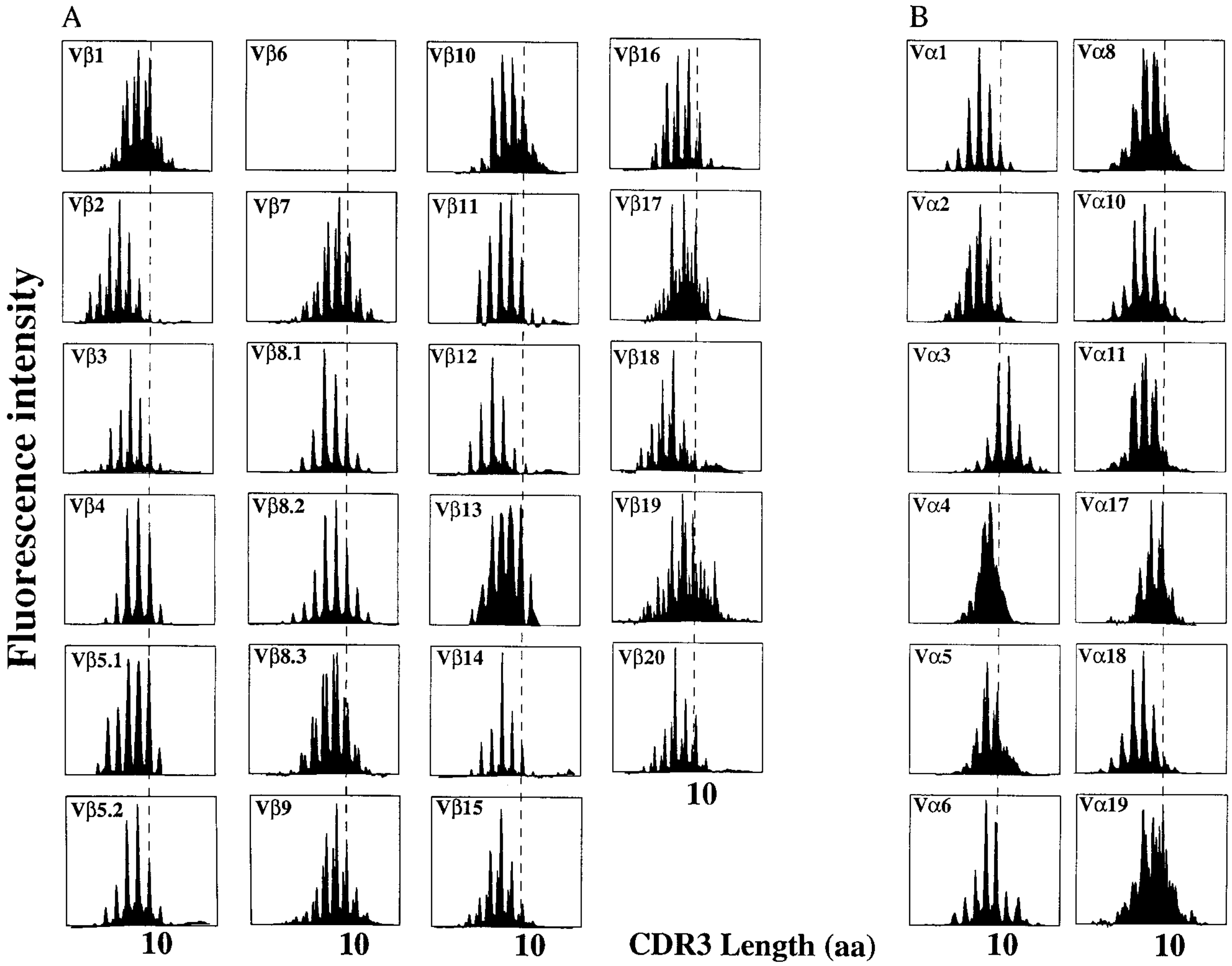

Figure 1. Profiles of the fluorescent Vβ-Cβ (*A*) and Vα-Cα (*B*) runoff products obtained with $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes from single chain peptide–MHC class II complex Tg. Results are representative of two mice tested independently. The intensity of fluorescence is represented in arbitrary units as a function of the size of the single DNA fragments.

CDR3 distribution of these rearrangements has a Gaussian-like profile (Vβ11 in Fig. 2 *B*; otherwise, data not shown). We have observed previously that in every situation characterized by the expansion of specific clone(s) (35, 36, 42) or the presence of oligoclonal populations (43), the analysis with the Immunoscope technique always revealed perturbations of the CDR3 size profile. Therefore, the Gaussian-like profile obtained for $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes selected by a single peptide–MHC class II complex indicates that the repertoire is polyclonal.

Comparison of the α/β Repertoire of $CD4^+CD8^-NK1.1^-HSA^-$ Thymocytes from Transgenic Mice for the Eα52-68 Peptide–I-A^b Complex and C57Bl/6 Mice. Repertoire analysis was performed on $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes from C57Bl/6 mice as described in previous sections. CDR3 size distributions obtained with Cβ or Cα primers for each Vβ or Vα chain were compared with those from cells selected by the single peptide–MHC class II complex (Fig. 2 *A* and data not shown). Our results show that for all Vβ and Vα, the profiles are superimposable. On Fig. 2 *A* are shown representative results for Vβ4, Vβ8.2, Vβ10, Vβ11, Vβ12, and Vβ14.

A more detailed analysis of Vβ-Jβ rearrangements was performed for Vβ4, Vβ8.2, Vβ10, Vβ11, Vβ12, and Vβ14. Representative results obtained with Vβ11 are shown on Fig. 2 *B*. The repertoires were superimposable and no significant modification was found with any Jβ primer.

Similar Usage of Jβ Gene Segments by TCR from $CD4^+CD8^-NK1.1^-HSA^-$ Thymocytes Selected in Wild-type Mice or in Transgenic Animals Expressing a Single Peptide–MHC Class II Complex. The relative Jβ usage in the Vβ11, Vβ12, and Vβ14 T cell population measured from one individual mouse of each strain (B2L and C57Bl/6) is shown in Fig. 3. The ratio of the area of the peaks generated with a given Jβ primer to the areas of all the Jβ primers was calculated as a measure of the relative frequency of Jβ usage. This analysis does not reveal any significant evidence of selection for a particular Jβ and shows that the Jβ2 segments are used more frequently than the Jβ1 segments as previously de-

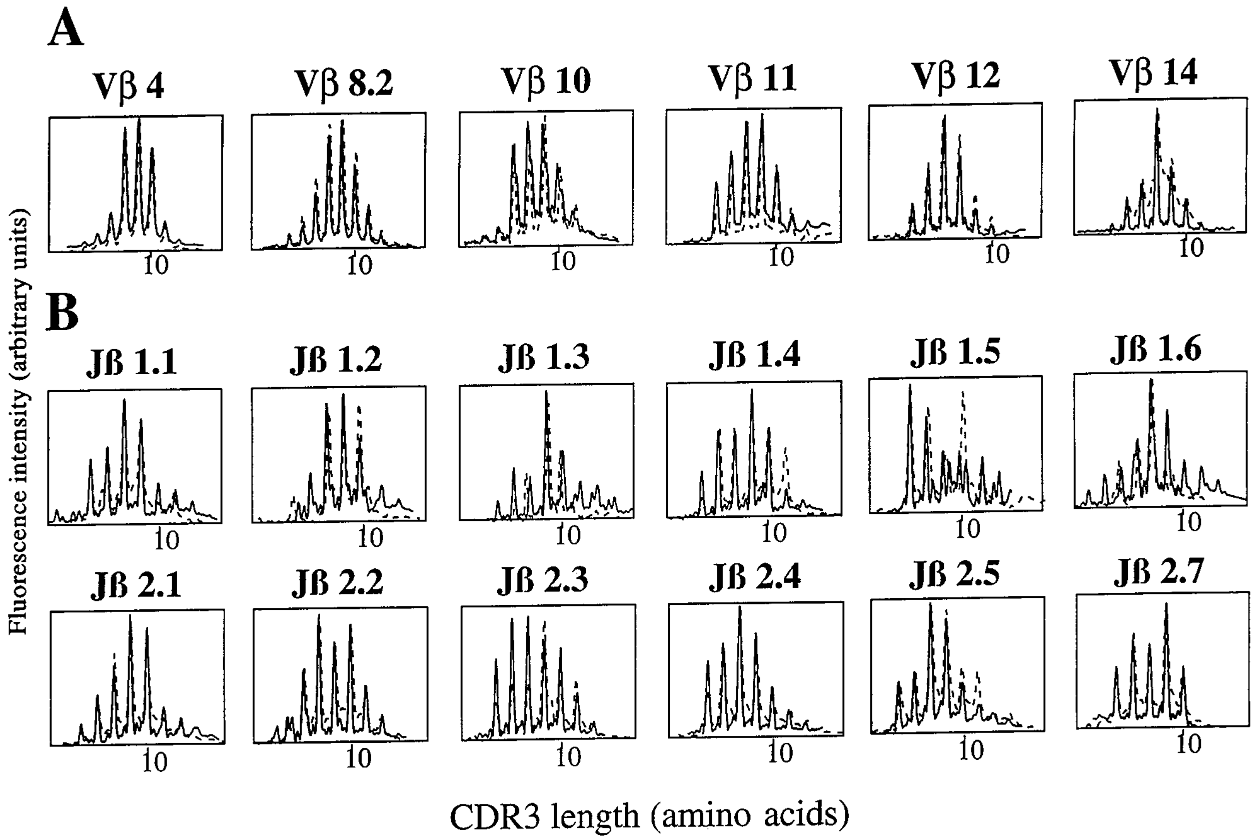

Figure 2. Comparison of the fluorescent runoff products profiles obtained from $CD4^+$ mature thymocytes from single chain peptide–MHC class II complex Tg (*solid lines*) and C57Bl/6 mice (*dotted lines*). Runoff extensions were performed for Vβ4, Vβ8.2, Vβ10, Vβ11, Vβ12, and Vβ14 with internal Cβ-specific fluorescent primer (*A*) and for Vβ11 with 12 Jβ-specific primers (*B*). The intensity of fluorescence is represented in arbitrary units as a function of CDR3 size in amino acids. Two mice were tested independently for each repertoire. Results were superimposable for the two mice tested in each combination.

scribed in the normal repertoire (44). Altogether, these results strongly suggest that the T cell repertoire selected by a single peptide–MHC complex is indistinguishable in terms of Vβ, Jβ, and Vα usage and CDR3 size distribution from the one selected by multiple peptide–MHC class II complexes.

Sequence Complexity in a Given Vβ-Jβ Rearrangement with a Defined CDR3 Length. Each peak corresponding to a given CDR3 length contains multiple distinct sequences. To evaluate the sequence complexity of the repertoire from $CD4^+$ T cells selected by the Eα52-68 peptide–I-A^b complex, we have chosen to focus arbitrarily on two particular rearrangements with a given CDR3 length: Vβ11-Jβ1.1 with a CDR3 length of six amino acids and Vβ12-Jβ1.1 with a CDR3 length of eight amino acids. These two Vβs are well selected in B2L mice, but reasonably abundant in both B2L and C57Bl/6 animals so that the anticipated complexity would be more amenable to our analysis. The same consideration guided the choice of the CDR3 lengths.

Each peak corresponding to these rearrangements was isolated and purified. The sequence mixture contained in these peaks was cloned and every positive clone was sequenced. More than 700 sequences were collected. We found that, in the two rearrangements tested, the number of different sequences collected from each sample is in the same range of magnitude, with a mean of 30 different sequences in the Vβ11-Jβ1.1 peak with a CDR3 length of six amino acids and a mean of 70 different sequences in the Vβ12-Jβ1.1 peak with a CDR3 length of eight amino acids (Table 1). Hence, sequence diversity contained in a given Vβ rearrangement with a particular length is the same between wild-type mice and Tg. Since we could estimate the number of different sequences contained in a single peak, we calculated the minimal number of rearrangements positively selected by the Eα52-68–I-A^b complex. The Vβ11-Jβ1.1 combination with a CDR3 length of six amino acids represents 13% of the rearrangements using the Vβ11 and Jβ1.1 gene segments. The Jβ1.1 is used in 5% of the rearrangements involving the Vβ11 gene segment. Staining with specific monoclonal antibody shows that 5% of the $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes from the Tg bear the Vβ11 chain (26). We then estimated that a minimum of 10^5 TCR-β rearrangements (30 × 7.7 [to correct for the CDR3 length] × 20 [to correct for all the Jβs] × 20 [to correct for all the Vβs]) are selected by the peptide–

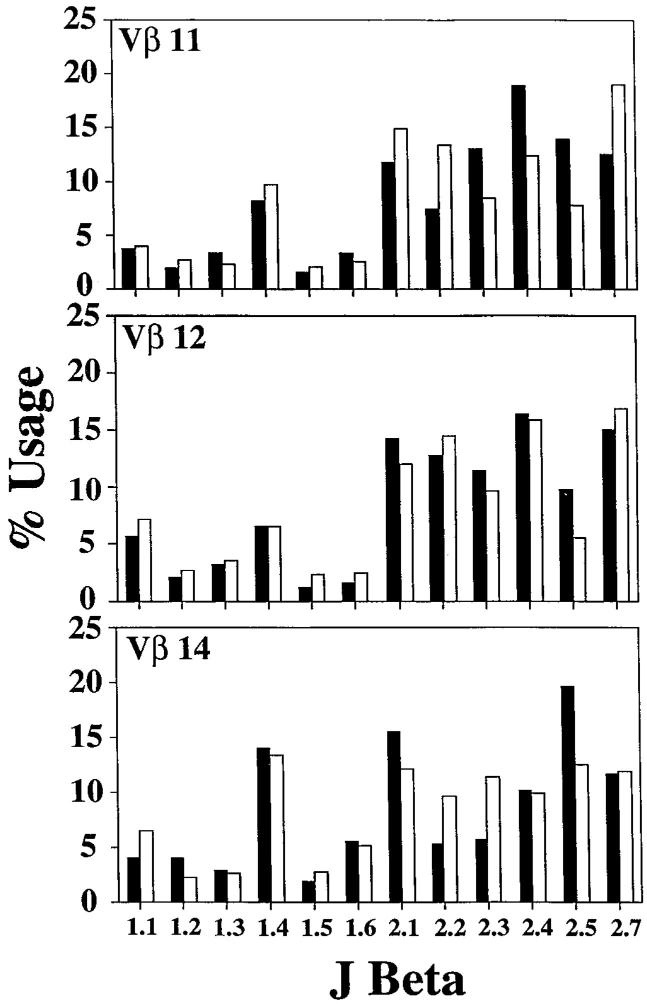

Figure 3. Jβ usage of Vβ11, Vβ12, and Vβ14 of $CD4^+CD8^-$ $NK1.1^-HSA^-$ thymocytes from B2L Tg (*black bars*) and C57Bl/6 mice (*white bars*). In the abscissa are represented all members of the Jβ family. In the ordinate is plotted the relative frequency of Jβ usage, calculated as described in Materials and Methods. The results were generated from one mouse of each strain.

MHC class II complex. The same calculation was applied for the Vβ12-Jβ1.1 rearrangement and a total of 7×10^4 TCR-β rearrangements were found.

Recurrent Sequences of the Vβ Chain CDR3 Region from $CD4^+$ $CD8^-NK1.1^-HSA^-$ Thymocytes of Wild-type Mice or Transgenic Animals Expressing Eα52-68–I-A^b Complex. Among the 700 sequences collected, we found some recurrent sequences between animals. The results obtained with the Vβ11-Jβ1.1 and Vβ12-Jβ1.1 rearrangements are shown in Fig. 4. For the Vβ11-Jβ1.1 rearrangement, only one recurrent amino acid sequence was found in all animals. This CDR3 is generated by the same nucleotide sequence and is encoded by the germline segments without trimming or N nucleotide addition (data not shown). For the Vβ12-Jβ1.1, four recurrent sequences were found between the C57Bl/6 mouse and one Tg, and seven between the same C57Bl/6 mouse and another Tg. Between the two Tg, nine amino

Table 1. *Sequence Complexity in a Given Vβ-Jβ Rearrangement with a Defined CDR3 Length*

Mice	Total No. of sequences	No. of different sequences	No. of unique sequences
Vβ11-Jβ1.1 CDR3 length: six amino acids			
C57Bl/6	159	34	12
C57Bl/6	180	27	0
Tg	33	25	18
Tg	58	34	21
Vβ12-Jβ1.1 CDR3 length: eight amino acids			
C57Bl/6	112	73	44
Tg	95	81	68
Tg	81	60	50

acid sequences of the CDR3 were recurrent. Among these, two CDR3 amino acid sequences were common to all animals tested: SLGANTEV and SLTANTEV (see Fig. 4). The SLGANTEV sequence was found one time in the C57Bl/6 animal, four times in one Tg, and two times in the other Tg tested (Table 2). Nucleotide sequence analysis of these rearrangements revealed that in each mouse, the sequence was generated differently by the recombination machinery (see Table 2). From the C57Bl/6 mice, the coding sequence was obtained with the trimming of three nucleotides from the Vβ12 germline segment, the usage of four nucleotides from the Dβ1 segment, 1 P nucleotide addition between the D and the J segments, and the germline sequence of the Jβ1.1 gene segment. This specific rearrangement was never found in the two Tg tested. In these two animals, recombination events (Vβ trimming, Dβ usage, N nucleotide addition) were different (see Table 2). However, one should notice that recurrent nucleotide sequences were also found between Tg. Comparable conclusions were reached when we analyzed the nucleotide sequences encoding the recurrent SLTANTEV (Table 2). Altogether, these results strongly suggest that these two CDR3 regions are not preferentially generated by the recombination machinery, but are positively selected at the amino acid level.

Comparison of the Amino Acid Frequency at Different Positions in the CDR3 Region of Vβ-Jβ Rearrangements from $CD4^+CD8^-NK1.1^-HSA^-$ Thymocytes of Wild-type Mice or Transgenic Animals Expressing Eα52-68–I-A^b Complex. We compared the amino acid usage in the CDR3 region of the two sequenced rearrangements Vβ11-Jβ1.1 (CDR3 length of six amino acids) and Vβ12-Jβ1.1 (CDR3 length of eight amino acids) of the $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes from Tg and wild-type mice. No difference was detected between mice when the individual percentage of amino acids in the CDR3 was plotted (data not shown). However, interesting differences became apparent when we plotted the frequency of individual amino acids at each position in the CDR3 loop (Figs. 5 and 6). Frequencies were calcu-

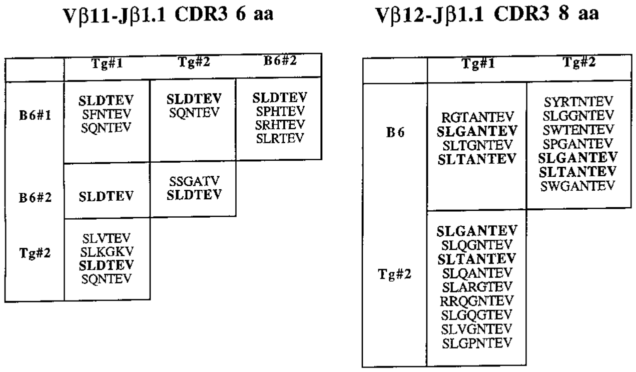

Figure 4. Recurrent sequences of the Vβ chain CDR3 region from $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes of wild-type mice or transgenic animals expressing Eα52-68–I-A^b complex are displayed. Recurrent sequences between individual animals are shown and the sequences common to all animals are in bold.

lated with nonredundant sequences in order to eliminate bias due to overrepresented sequences. Positions 2 and 3 for the Vβ11-Jβ1.1 rearrangement and 2, 3, and 4 for the Vβ12-Jβ1.1 rearrangement are more variable due to N or P nucleotide additions and reading frame usage of the Dβ gene segment. One can notice that for each variable position, a limited number of amino acid residues are found and that they are similar in all mice. Furthermore, the percentage of amino acid residues found at each variable position (2, 3, and 4) is strikingly similar between C57Bl/6 mice and Tg. However, for the Vβ11-Jβ1.1 rearrangement, it appears that at position three of the CDR3 from Tg, there is an increase in the frequency of the asparagine residue as compared with wild-type mice (24 versus 8%; see Fig 5). Similar skewing is also found with the Vβ12-Jβ1.1 rearrangement. When comparing amino acid frequency between wild-type mice and Tg, we found an increase in leucine at position 2, threonine and glutamine at position 3, and glycine at position 4 of the CDR3 loop of transgenic animals (Fig. 6). In contrast, in C57Bl/6 mice, glycine is preferentially found in position 3 of the CDR3. Analysis of nucleotide sequences encoding glycine or glutamine at this position shows that both amino acids are encoded by the Dβ1.1 gene segment in all CDR3s. Glycine can be encoded by all three Dβ1.1 reading frames. There are six possibilities to code for glycine. In Tg and wild-type mice, all encoding possibilities are used (Fig. 7), indicating that there is no bias due to the recombination machinery between these mice. Strikingly, in Tg animals, glutamine is found more frequently at this position (Fig. 6) even though this residue is only encoded by frame 2 of the Dβ1.1 gene segment. Altogether these results suggest that this residue has been selected for at the amino acid level. Furthermore, the preferential usage of the Dβ1.1 frame 2 in the transgenic animals should favor the appearance of a glycine residue at position 4. This increase in glycine frequency at position 4 is observed in transgenic animals and not in C57Bl/6 (Fig. 6). These differences in amino acid usage at different positions of the β chain CDR3 junctions reveal the imprint of the Eα52-68 peptide–I-A^b complex on the selected T cell repertoire.

Table 2. *Nucleotide and Amino Acid Translated Sequences of Vβ12-Jβ1.1 Recurrent Rearrangements with a CDR3 Length of Eight Amino Acids*

Germline	Vβ12	N/P	Dβ1	N/P	Jβ1.1	
	TGT GCC AGC AGT TTA GC		GGG ACA GGG GGC		CA AAC ACA GAA GTC TTC	
C57Bl/6						
1×*	TGT GCC AGC AGT TT		GGG A	G	CA AAC ACA GAA GTC TTC	CASSLGANTEV
Tg1						
1×	TGT GCC AGC AGT TTA G		GGG		CA AAC ACA GAA GTC TTC	CASSLGANTEV
3×	TGT GCC AGC AGT TTA G		G GGC	C	AAC ACA GAA GTC TTC	CASSLGANTEV
Tg2						
2×	TGT GCC AGC AGT TTA G		GGG		CA AAC ACA GAA GTC TTC	CASSLGANTEV
C57Bl/6						
1×	TGT GCC AGC AGT TTA		ACA G		CA AAC ACA GAA GTC TTC	CASSLTANTEV
Tg1						
2×	TGT GCC AGC AGT TT		G ACA G		CA AAC ACA GAA GTC TTC	CASSLTANTEV
Tg2						
1×	TGT GCC AGC AGT TTA		ACA G		CA AAC ACA GAA GTC TTC	CASSLTANTEV
1×	TGT GCC AGC AGT TTA		AC	GG	CA AAC ACA GAA GTC TTC	CASSLTANTEV
3×	TGT GCC AGC AGT TT		G ACA G		CA AAC ACA GAA GTC TTC	CASSLTANTEV

*No. of times this sequence was found in the animal.

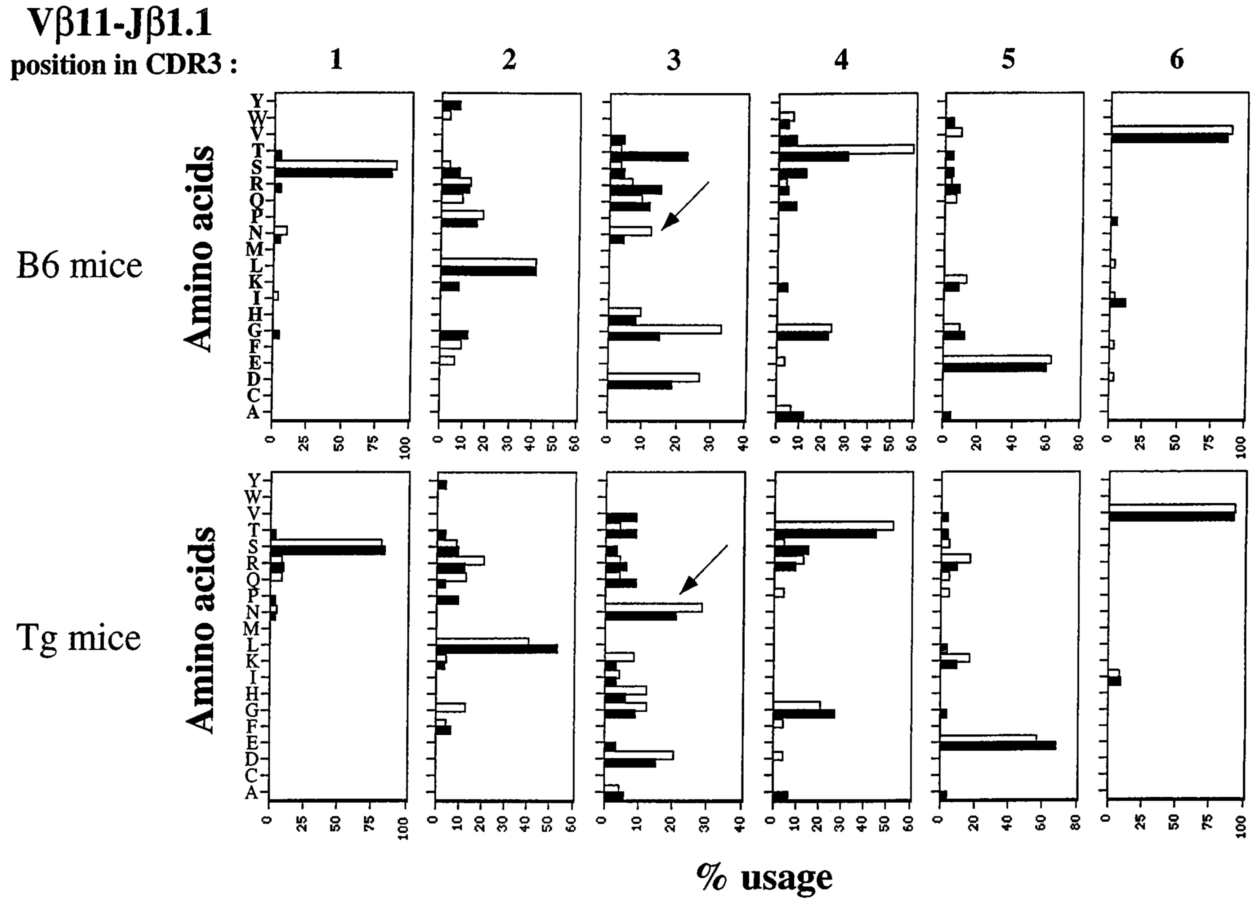

Figure 5. Amino acid frequency at the different positions in the CDR3 region of Vβ11-Jβ1.1 (length: six amino acids) rearrangements from $CD4^+CD8^-NK1.1^-HSA^-$ thymocytes. *Top,* the results obtained from two independent C57Bl/6 mice; *bottom,* the results obtained from two independent Tg. Amino acid frequencies were calculated with nonredundant sequences to avoid skewing due to overrepresented sequences (34 and 27 sequences and 25 and 34 sequences were used for C57Bl/6 mice and Tg, respectively).

Discussion

At Least 10^5 Distinct TCR-β Rearrangements Are Selected by the Single MHC–Peptide Complex Displayed in B2L Mice. The involvement of peptides presented by MHC molecules in the process of thymic positive selection of T cells has been investigated in vivo (25, 26, 45–49) and in vitro (11, 12, 14, 50). Recent studies (23, 24, 27) have shown that positive selection is a promiscuous process enabling the selection of many different TCR rearrangements by a single peptide–MHC class II complex, but their diversity has not been quantified so far. Here, we have estimated the number of TCR rearrangements in mature thymocytes positively selected in Tg B2L mice (26) designed to express a single peptide–MHC class II complex (the Eα52-68 peptide hooked to $I\text{-}A^b$). Their $Ii^{-/-}$ genetic background guarantees that the physiological route of peptide loading is blocked (29). Similar mice, engineered by Ignatowicz et al. (25), have been shown (contrary to $H\text{-}2M^{-/-}$ mice) to present no other detectable self-peptides (22).

B2L mice express low levels of the $E\alpha52\text{-}68\text{–}I\text{-}A^b$ complex in their thymus and on ~5–10% of their splenocytes and positively select a number of $CD4^+$ T cells, which is ~20–50% of C57Bl/6 (26). We analyzed RNA from $CD4^+CD8^-NK1.1^-HSA^-$ T cells isolated from C57Bl/6 and from B2L mice by a PCR-based approach designed to determine the CDR3 lengths of the β and α chains. All Vβ-Jβ combinations were used to visualize an overall picture of the β repertoire based upon some 2,000 measurements. Given the larger number of Jα segments, the α repertoire was not totally analyzed but sampled with 12 Vα families. The normal mouse repertoire is characterized by bell-shaped, Gaussian-like, CDR3 length distributions (30). Distortions are observed upon antigenic stimulations that cause sufficient clonal expansion (34–36, 42). Oligoclonal distributions in pathological infiltrates are easily depicted (51–55). B2L $CD4^+$ T cell profiles were remarkably Gaussian-like and quasisuperimposable to wild type. The Jβ usage for Vβ11, Vβ12, and Vβ14 closely matched that observed in C57Bl/6. Moreover, B2L and C57Bl/6 displayed overlapping CDR3 size distributions, even though the average CDR3 length is different for distinct Vβ segments (30). No spikes suggesting clonal or oligoclonal expansions were detected. Therefore, the single MHC–pep-

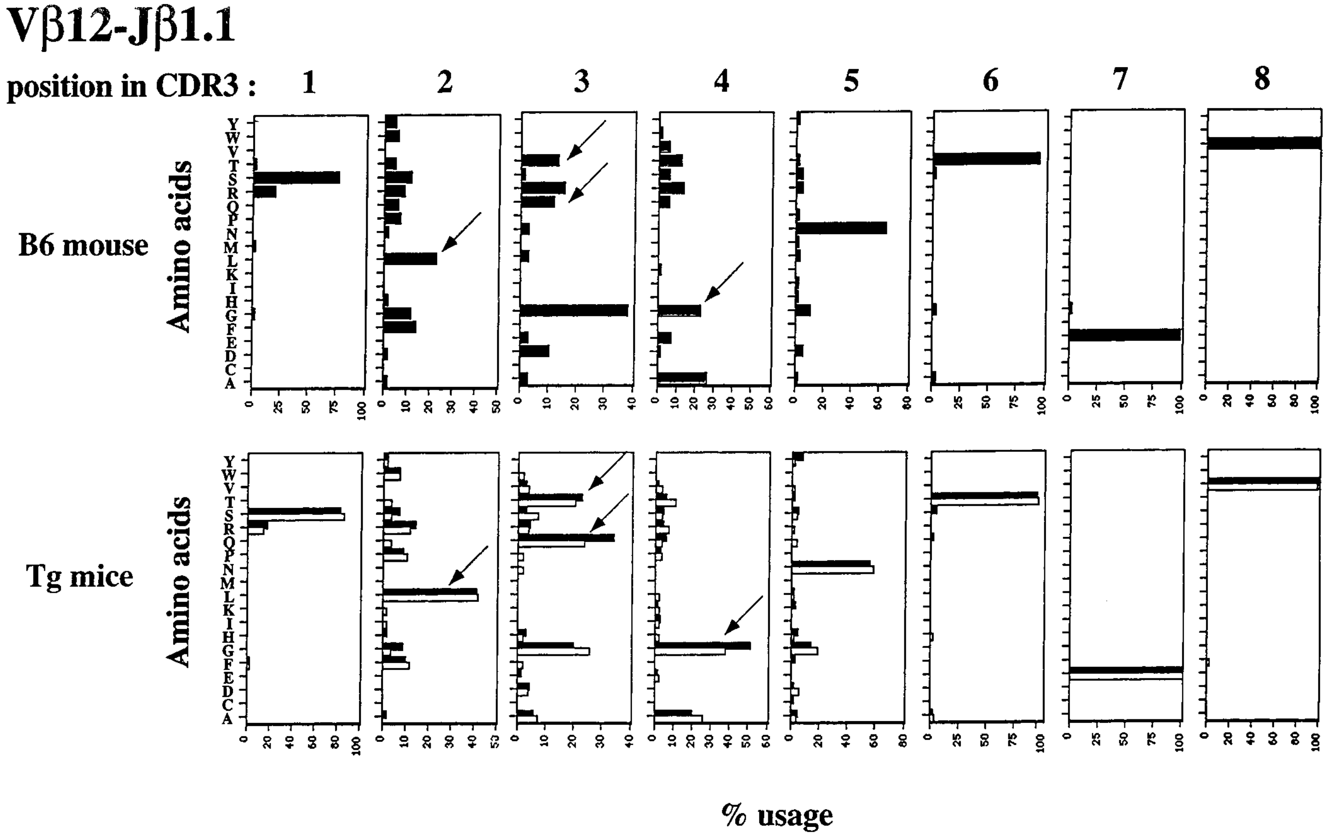

Figure 6. Amino acid frequency at the different positions in the CDR3 region of Vβ12-Jβ1.1 (length: eight amino acids) rearrangements from CD4$^+$CD8$^-$NK1.1$^-$HSA$^-$ thymocytes. *Top*, the results obtained from one C57Bl/6 mouse; *bottom*, the percentage of usage obtained from two independent Tg. Amino acid frequencies were calculated with nonredundant sequences to avoid skewing due to overrepresented sequences (73 sequences and 81 and 60 sequences were used for C57Bl/6 mice and Tg, respectively).

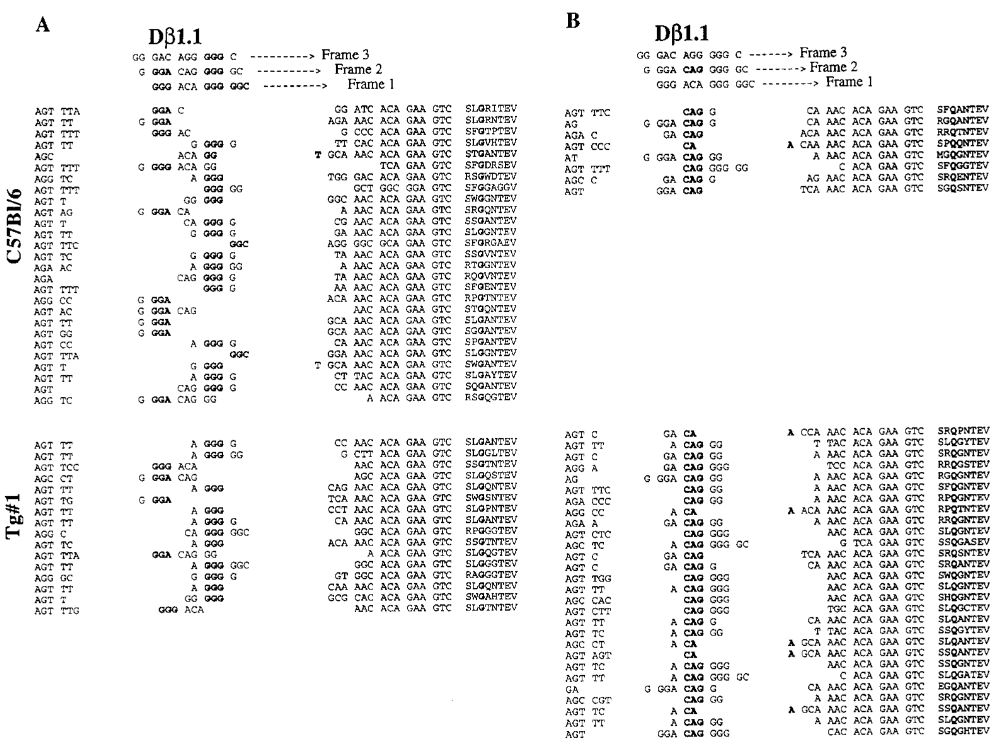

Figure 7. Dβ reading frame usage coding for glycine or glutamine residues at position 3 of eight amino acids–long CDR3 region of Vβ12-Jβ1.1 rearrangements. (*A*) The nucleotide sequences (*bold*) encoding a glycine residue at position 3 of the CDR3 region from C57Bl/6 (*top*) and Tg (*bottom*) thymocytes. (*B*) The nucleotide sequences (*bold*) encoding a glutamine residue at position 3 of the CDR3 region from C57Bl/6 (*top*) and Tg (*bottom*) thymocytes. All the sequences were aligned following the Dβ reading frame usage, regardless of the Vβ, Jβ gene segment trimming, and N addition.

tide complex in B2L mice did not preferentially select a small number of clones over an otherwise polyclonal background.

We cloned and sequenced from B2L and C57Bl/6 mice the Vβ11-Jβ1.1 and Vβ12-Jβ1.1 size peaks with CDR3 lengths of six and eight amino acids, respectively. We could thus (Table 1) establish that the Tg and wild-type mice repertoires both include a minimum of 10^5 β rearrangements, suggesting that the Tg repertoire is about as diverse as the wild-type one. In addition, the number of distinct α chains capable of pairing with a given rearranged β chain has not been determined in physiological conditions. The work of Sant'Angelo et al. (56) showed that 20–30 different α chains can associate with a unique transgenic rearranged β chain. If this figure can be extrapolated to physiological situations, the number of different TCRs would be much higher than 10^5, leaving open the possibility that each positively selected $CD4^+$ thymocyte displays a unique TCR.

The Transgenic Repertoire Is Skewed in at least Two Ways with Respect to the Wild-type Repertoire. The above data show that the preferential selection of certain Vβs, observed in B2L mice by Fukui et al. (26), is not reflected in a few clonal expansions but in a larger number of rearrangements as revealed by the Gaussian distributions. This suggests that the Eα52-68 peptide prevents proper association with the subset of Vβ segments that are not efficiently selected, that it is instrumental in binding those Vβ segments that are selected (57), or both. The amino acid usage, compiled in Figs. 5 and 6, shows a second level of skewing now involving the CDR3 regions. We are confident that these differences in the amino acid composition at some CDR3 positions are significant. First, in conserved positions we find no variation of the amino acid usage between Tg and wild-type mice. Second, we evaluated the percentage of sequencing errors at conserved positions such as the $C92^-$, $A93^-$,$S94^-$-encoded positions of the CDR3. The figure of 0.3% at each position cannot explain the results. Third, amino acid usage calculations were done with nonredundant sequences in order to avoid skewing due to overrepresentation. Fourth, such percentage variations are consistent from one mouse to another of the same lineage (Tg versus wild-type mice).

The elegant studies by Sant'Angelo et al. (56) have provided strong evidence for an imprint of the peptide in the process of positive selection. Here we had no direct way to evaluate the respective impact of positive and negative selection in shaping the B2L repertoire of $CD4^+$ thymocytes. However, the recurrent β chain rearrangements that we have observed (Fig. 4) are likely to reflect positive selection events, since they could hardly be the result of chance, or of a negative process that would delete all sequences but these ones. Remarkably, among these recurrent β chains, two were found in both B2L and C57Bl/6. They were encoded by nonidentical nucleotide sequences and generated by distinct junctional events (Figs. 4 and 7), implying that they were selected at the amino acid level in a positive fashion. It is worth noting that C57Bl/6 mice do not express nor present the Eα52-68 peptide (58, 59). Therefore, in this case, the event that positively selected the β chain involved no peptide or self-peptides sharing amino acid residues with Eα52-68, or was mediated by the α chain.

In the peripheral response to several defined antigens, one or a few specific CDR3 sequences, in given Vβ-Jβ and/or Vα-Jα combinations, have been found to be highly reproducible and shared by individual animals. They were named "public" (35) by analogy with recurrent idiotypes found in immunoglobulins (60). Whether these public rearrangements are selected in the periphery or in the thymus has not been determined so far. Since, as shown above, at least some recurrent rearrangements are positively selected in the thymus, it may be proposed that peripheral public rearrangements, in general, bear the imprint of thymic positive selection.

Altogether, our results also show that the impact of a peptide in positive selection may not be readily detected. First, our data indicate that the peptide may not be directly involved in all selection events regarding the β chain since the α chain may also be involved in the selection process. This raises the possibility that not all TCR-β rearrangements bear the imprint of the peptide. Second, in order to detect the latter, we had to focus on a specific Vβ-Jβ combination with a fixed CDR3 length, whereas Sant'Angelo et al. studied α chain rearrangements associated with a β chain that had been fixed by transgenesis (56). This may explain some apparently conflicting results about peptide specificity in positive selection.

How is the T Cell Repertoire Shaped in Wild-type Mice? The physiological relevance of observations made in transgenic animals is questionable. Among the few reports on positive selection in nontransgenic animals (for review see reference 61), the comparison of positive selection of anti-ovalbumin TCR in K^b and K^{bm8} mutant mice has provided a clear indication for the involvement of presented peptides (45). The single peptide–MHC complex of B2L mice makes up ∼10% of physiological complexes in the thymic cortex of B10-A(5R) mice. According to Grubin et al. (22), rare peptides (expressed, in their case, in the H-2M–deficient background) appear functional in positive selection whether the diversity of the T cell repertoire is built up only by the most abundant self-peptides like Eα52-68, or by rarer self-peptides as well. In the latter case, it remains to be determined to which extent the diversity of thymocytes is increased by rarer peptides in a cumulative fashion. We are currently performing an extensive repertoire analysis of B10-A(5R) mice in order to evaluate whether and to which degree it includes or overlaps with that of B2L animals.

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References

1. Kappler, J.W., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. *Cell.* 49:273–280.
2. Kisielow, P., H. Blüthmann, U.D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell–receptor transgenic mice involves deletion of nonmature $CD4^+8^+$ thymocytes. *Nature.* 333:742–746.
3. Kisielow, P., H.S. Teh, H. Blüthmann, and H. von Boehmer. 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature.* 335:730–733.
4. Scott, B., H. Blüthmann, H.S. Teh, and H. von Boehmer. 1989. The generation of mature T cells require interaction of the $\alpha\beta$ T-cell receptor with major histocompatibility antigens. *Nature.* 338:591–593.
5. Zinkernagel, R.M., and P.C. Doherty. 1974. Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. *Nature.* 251: 547–548.
6. Bevan, M.J. 1977. In a radiation chimaera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells. *Nature.* 269:417–418.
7. Zinkernagel, R.M., G. Callahan, A. Althage, S. Cooper, P. Klein, and J. Klein. 1978. On the thymus in the differentiation of H-2 self-recognition by T cells: evidence for dual recognition. *J. Exp. Med.* 147:882–896.
8. Fink, P.J., and M.J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* 148:766–775.
9. MacDonald, H.R., T. Pedrazzini, R. Schneider, J.A. Louis, R.M. Zinkernagel, and H. Hengartner. 1988. Intrathymic elimination of Mlsa-reactive (V beta 6+) cells during neonatal tolerance induction to Mlsa-encoded antigens. *J. Exp. Med.* 167:2005–2010.
10. Sha, W.C., C.A. Nelson, R.D. Newberry, D.M. Kranz, J.H. Russell, and D.Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature.* 336:73–76.
11. Ashton-Rickardt, P.G., L. van Kaer, T.N.M. Schumacher, H.L. Ploegh, and S. Tonegawa. 1993. Peptide contributes to the specificity of positive selection of $CD8^+$ T cells in the thymus. *Cell.* 73:1041–1049.
12. Hogquist, K.A., M.A. Gavin, and M.J. Bevan. 1993. Positive selection of $CD8^+$ T cells induced by major histocompatibility complex binding peptides in fetal thymic organ culture. *J. Exp. Med.* 177:1469–1473.
13. Hogquist, K.A., S.C. Jameson, and M.J. Bevan. 1994. The ligand for positive selection of T lymphocytes in the thymus. *Curr. Opin. Immunol.* 6:273–278.
14. Ashton-Rickardt, P.G., A. Bandeira, J.R. Delaney, L. van Kaer, H.-P. Pircher, R.M. Zinkernagel, and S. Tonegawa. 1994. Evidence for a differential avidity model of T cell selection in the thymus. *Cell.* 76:651–663.
15. Sebzda, E., V.A. Wallace, J. Mayer, R.S.M. Yeung, T.W. Mak, and P.S. Ohashi. 1994. Positive and negative thymocyte selection induced by different concentrations of a single peptide. *Science.* 263:1615–1618.
16. Hogquist, K.A., S.C. Jameson, and M.J. Bevan. 1995. Strong agonist ligands for the T cell receptor do not mediate positive selection of functional CD8+ T cells. *Immunity.* 3:79–86.
17. Alam, S.M., P.J. Travers, J.L. Wung, W. Nasholds, S. Redpath, S.C. Jameson, and N.R.J. Gascoigne. 1996. T-cell–receptor affinity and thymocyte positive selection. *Nature.* 381:616–620.
18. Hogquist, K.A., A.J. Tomlinson, W.C. Kieper, M.A. McGargill, M.C. Hart, S. Naylor, and S.C. Jameson. 1997. Identification of a naturally occurring ligand for thymic positive selection. *Immunity.* 6:389–399.
19. Hu, Q., C.R. Bazemore Walker, C. Girao, J.T. Opferman, J. Shabanowitz, D.F. Hunt, and P.G. Ashton-Rickardt. 1997. Specific recognition of thymic self-peptides induces the positive selection of cytotoxic lymphocytes. *Immunity.* 7:221–231.
20. Miyazaki, T., P. Wolf, S. Tourne, C. Waltzinger, A. Dierich, N. Barois, H. Ploegh, C. Benoist, and D. Mathis. 1996. Mice lacking H2-M complexes, enigmatic elements of the MHC class II peptide-loading pathway. *Cell.* 84:531–541.
21. Martin, D.W., G.G. Hicks, S.K. Mendiratta, H.I. Leva, H.E. Ruley, and L. Van Kaer. 1996. H2-M mutant mice are defective in the peptide loading of class II molecules, antigen presentation, and T cell repertoire selection. *Cell.* 84:543–550.
22. Grubin, C.E., S. Kovats, P. deRoos, and A.Y. Rudensky. 1997. Deficient positive selection of CD4 T cells in mice displaying altered repertoires of MHC class II–bound self-peptides. *Immunity.* 7:197–208.
23. Surh, C.D., D.-S. Lee, W. Fung-Leung, L. Karlsson, and J. Sprent. 1997. Thymic selection by a single MHC/peptide ligand produces a semidiverse repertoire of CD4+ T cells. *Immunity.* 7:209–219.
24. Tourne, S., T. Miyazaki, A. Oxenius, L. Klein, T. Fehr, B. Kyewski, C. Benoist, and D. Mathis. 1997. Selection of a broad repertoire of $CD4^+$ T cells in $H\text{-}2Ma^{\circ/\circ}$ mice. *Immunity.* 7:187–195.
25. Ignatowicz, L., J. Kappler, and P. Marrack. 1996. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell.* 84:521–529.
26. Fukui, Y., T. Ishimoto, M. Utsuyama, T. Gyotoku, T. Koga, K. Nakao, K. Hirokawa, M. Katsuki, and T. Sasazuki. 1997. Positive and negative $CD4^+$ thymocyte selection by a single MHC class II/peptide ligand in vivo affected by its expression

level in the thymus. *Immunity*. 6:401–410.
27. Ignatowicz, L., W. Rees, R. Pacholczyk, H. Ignatowicz, E. Kushnir, J. Kappler, and P. Marrack. 1997. T cells can be activated by peptides that are unrelated in sequence to their selecting peptide. *Immunity*. 7:179–186.
28. Cosgrove, D., D. Gray, A. Dierich, J. Kaufman, M. Lemeur, C. Benoist, and D. Mathis. 1991. Mice lacking MHC class II molecules. *Cell*. 66:1051–1066.
29. Viville, S., J. Neefjes, V. Lotteau, A. Dierich, M. Lemeur, H. Ploegh, C. Benoist, and D. Mathis. 1993. Mice lacking the MHC class II–associated invariant chain. *Cell*. 72:635–648.
30. Pannetier, C., M. Cochet, S. Darche, A. Casrouge, M. Zoller, and P. Kourilsky. 1993. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. *Proc. Natl. Acad. Sci. USA*. 90:4319–4323.
31. Casanova, J.L., P. Romero, C. Widmann, P. Kourilsky, and J.L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex–restricted cytotoxic T lymphocyte clones specific for a *Plasmodium berghei* nonapeptide: implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med*. 174:1371–1383.
32. Pannetier, C., J. Even, and P. Kourilsky. 1995. T-cell repertoire diversity and clonal expansions in normal and clinical samples. *Immunol. Today*. 16:176–181.
33. Pannetier, C., J.-P. Levraud, J. Even, and P. Kourilsky. 1996. The Immunoscope technique for analysis of TCR repertoire. *In* The Human Antigen T Cell Receptor. Selected Protocols and Applications. J.R. Oksenberg, ed. Landes, R.G. Publishing Co., Austin, TX. 287–325.
34. Cochet, M., C. Pannetier, A. Regnault, S. Darche, C. Leclerc, and P. Kourilsky. 1992. Molecular detection and *in vivo* analysis of the specific T cell response to a protein antigen. *Eur. J. Immunol*. 22:2639–2642.
35. Cibotti, R., J.P. Cabaniols, C. Pannetier, C. Delarbre, I. Vergnon, J.M. Kanellopoulos, and P. Kourilsky. 1994. Public and private V beta T cell receptor repertoires against hen egg white lysozyme (HEL) in nontransgenic versus HEL transgenic mice. *J. Exp. Med*. 180:861–872.
36. Levraud, J.P., C. Pannetier, P. Langlade-Demoyen, V. Brichard, and P. Kourilsky. 1996. Recurrent T cell receptor rearrangements in the cytotoxic T lymphocyte response in vivo against the p815 murine tumor. *J. Exp. Med*. 183:439–449.
37. Musette, P., A. Galelli, P. Truffa-Bachi, W. Peumans, P. Kourilsky, and G. Gachelin. 1996. The Jb segment of the T cell receptor contributes to the Vβ-specific T cell expansion caused by staphylococcal enterotoxin B and *Urtica dioica* superantigens. *Eur. J. Immunol*. 26:618–622.
38. Bendelac, A. 1995. Positive selection of mouse $NK1^+$ T cells by CD1-expressing cortical thymocytes. *J. Exp. Med*. 182: 2091–2096.
39. Kappler, J., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, R. Roehm, and P. Marrack. 1987. A T cell receptor Vβ segment that imparts reactivity to a class II major histocompatibility complex product. *Cell*. 49:263–269.
40. Louie, M.C., C.A. Nelson, and D.Y. Loh. 1989. Identification and characterization of new murine T cell receptor β chain variable region (Vβ) genes. *J. Exp. Med*. 170:1987–1998.
41. Wade, T., J. Bill, P.C. Marrack, E. Palmer, and J.W. Kappler. 1988. Molecular basis for the nonexpression of vβ17 in some strains of mice. *J. Immunol*. 141:2165–2167.
42. Gapin, L., Y. Bravo de Alba, A. Casrouge, J.-P. Cabaniols, P. Kourilsky, and J. Kanellopoulos. 1998. Dendritic cells focus T-cell response against an immunodominant peptide: study in the hen egg-white lysozyme (HEL) model. *J. Immunol*. 160:1555–1564.
43. Regnault, A., A. Cumano, P. Vassalli, D. Guy-Grand, and P. Kourilsky. 1994. Oligoclonal repertoire of the CD8αα and the CD8αβ TCR-α/β murine intestinal intraepithelial T lymphocytes: evidence for the random emergence of T cells. *J. Exp. Med*. 180:1345–1358.
44. Candeias, S., C. Waltzinger, C. Benoist, and D. Mathis. 1991. The V beta 17+ T cell repertoire: skewed J beta usage after thymic selection; dissimilar CDR3s in CD4+ versus CD8+ cells. *J. Exp. Med*. 174:989–1000.
45. Nikolic-Zugic, J., and M.J. Bevan. 1990. Role of self-peptides in positively selecting the T-cell repertoire. *Nature*. 344: 65–67.
46. Sha, W.C., C.A. Nelson, R.D. Newberry, J.K. Pullen, L.R. Pease, J.H. Russell, and D.Y. Loh. 1990. Positive selection of transgenic receptor-bearing thymocytes by K^b antigen is altered by K^b mutations that involve peptide binding. *Proc. Natl. Acad. Sci. USA*. 87:6186–6190.
47. Jacobs, H., H. von Boehmer, C.J. Melief, and A. Berns. 1990. Mutations in the major histocompatibility complex class I antigen-presenting groove affect both negative and positive selection of T cells. *Eur. J. Immunol*. 20:2333–2337.
48. Ohashi, P.S., R.M. Zinkernagel, I. Leuscher, H. Hengartner, and H. Pircher. 1993. Enhanced positive selection of a transgenic TCR by a restriction element that does not permit negative selection. *Int. Immunol*. 5:131–138.
49. Nakano, N., R. Rooke, C. Benoist, and D. Mathis. 1997. Positive selection of T cells induced by viral delivery of neopeptides to the thymus. *Science*. 275:678–683.
50. Jameson, S.C., K.A. Hogquist, and M.J. Bevan. 1995. Positive selection of thymocytes. *Annu. Rev. Immunol*. 13:93–126.
51. Caignard, A., P.Y. Dietrich, V. Morand, A. Lim, C. Pannetier, A.M. Leridant, T. Hercend, J. Even, P. Kourilsky, and F. Triebel. 1994. Evidence for T-cell clonal expansion in a patient with squamous cell carcinoma of the head and neck. *Cancer Res*. 54:1292–1297.
52. Dietrich, P.Y., A. Caignard, A. Lim, V. Chung, J.L. Pico, C. Pannetier, P. Kourilsky, T. Hercend, J. Even, and F. Triebel. 1994. In vivo T-cell clonal amplification at time of acute graft-versus-host disease. *Blood*. 84:2815–2820.
53. Douillard, P., C. Pannetier, R. Josien, S. Menoret, P. Kourilsky, J.P. Soulillou, and M.C. Cuturi. 1996. Donor-specific blood transfusion induced tolerance in adult rats with a dominant TcR-V beta rearrangement in heart allografts. *J. Immunol*. 157:1250–1260.
54. Musette, P., D. Bequet, C. Delarbre, G. Gachelin, P. Kourilsky, and D. Dormont. 1997. Expansion of a recurrent Vβ5.3+ T cell population in newly diagnosed and untreated HLA-DR2 multiple sclerosis patients. *Proc. Natl. Acad. Sci*. 93:12461–12466.
55. Musette, P., J.F. Bureau, G. Gachelin, P. Kourilsky, and M. Brahic. 1995. T lymphocyte repertoire in Theiler's virus encephalomyelitis: the nonspecific infiltration of the central nervous system of infected SJL/J mice is associated with a selective local T cell expansion. *Eur. J. Immunol*. 25:1589–1593.
56. Sant'Angelo, D.B., P.G. Waterbury, B.E. Cohen, W.D. Martin, L. Van Kaer, A.C. Hayday, and C.A. Janeway, Jr. 1997. The imprint of intrathymic self-peptides on the mature T cell receptor repertoire. *Immunity*. 7:517–524.

57. Garboczi, D.N., P. Ghosh, Q.R. Fan, W.E. Biddison, and D.C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature.* 384: 134–141.
58. Rudensky, A., S. Rath, H.P. Preston, D.B. Murphy, and C.A. Janeway. 1991. On the complexity of self. *Nature.* 353: 660–662.
59. Murphy, D.B., R. Satyajit, E. Pizzo, A.Y. Rudensky, A. George, J.K. Larson, and C.A. Janeway, Jr. 1992. Monoclonal antibody detection of a major self peptide MHC class II complex. *J. Immunol.* 148:3483–3491.
60. Urbain, J., C. Wuilmart, and P.-A. Cazenave. 1981. Idiotypic regulation in immune networks. *Contemp. Top. Mol. Immunol.* 8:113–117.
61. Fink, P.J., and M.J. Bevan. 1995. Positive selection of thymocytes. *Adv. Immunol.* 59:99–133.