The $V_{\beta 17}^+$ T Cell Repertoire: Skewed J$\beta$ Usage after Thymic Selection; Dissimilar CDR3s in CD4$^+$ Versus CD8$^+$ Cells

By Serge Candéias, Caroline Waltzinger, Christophe Benoist, and Diane Mathis

From the Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l’INSERM, Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg France

Summary

To ascertain how the actual repertoire of T cell receptors (TCRs) deviates from the theoretical, we have generated a large number of junctional region sequences from TCRs carrying the $V_{\beta 17}$ variable region. The >600 sequences analyzed represent transcripts from nine different cell populations, permitting several comparisons: transcripts from an expressed vs. a non-expressed $V_{\beta 17}$ allele, those from E$^+$ vs. E$^-$ mice, transcripts from immature vs. mature thymocytes, those from thymic vs. peripheral T cells, and those from CD4$^+$ vs. CD8$^+$ cells. These comparisons have allowed us to distinguish between the influence of molecular events involved in TCR gene rearrangement and that of various selection events that shape the T cell repertoire. Our most striking findings are: (a) that J$\beta$ usage is markedly skewed, partly due to recombination mechanics and partly due to selection forces: in particular, those mediated by the class II E molecule in the thymus; and (b) that TCRs on CD4$^+$ and CD8$^+$ cells show intriguing dissimilarities. In addition, we present evidence that N nucleotide additions occur with clear biases, probably due to idiosyncrasies of the recombination enzymes, and provide arguments that TCR and immunoglobulin CDR3s have distinct structures.

Theoretically, the murine immune system can make use of an enormous repertoire of $\alpha/\beta$ T cells. Any rearranged TCR $\beta$ chain gene is created by the juxtaposition of 1 of 22 V, 1 of 2 D, and 1 of 12 J region segments (for reviews, see references 1 and 2). At each of the juxtaposed segment termini (3'V, 5'D, 3'D, 5'J) one or two so-called P nucleotides can be added, almost certainly as byproducts of the mechanics of rearrangement (3); some of the terminal nucleotides can then be nibbled away by an undefined exonuclease activity (1, 2). Finally, a variable number of so-called N nucleotides can be inserted at the V-D and D-J junctions by a template-independent mechanism (1, 2). Assuming that these processes operate randomly, and considering similar processes to be in play with the $\alpha$ chain genes, Davis and Bjorkman (4) have calculated that there may be as many as $10^{13}$ $\alpha/\beta$ TCR specificities available to the mouse.

But is this enormous repertoire actually realized? Already there have been some indications that it is not. Some mice lack particular V (5, 6) or J (7) gene segments, or do not express certain ones because of mutations (8). Some animals delete from the peripheral repertoire almost all T cells displaying particular V$\beta$s, seemingly because they are self reactive (for reviews, see references 9 and 10). In this report, we have sought to determine whether the junctional regions of TCR $\beta$ chains really have a haphazard constitution. According to Davis and Bjorkman (4), the TCR junctional regions should make by far the greatest contribution to their diversity. We have asked questions like: is J region usage entirely random? Is D region usage? Are P nucleotides found at random? What are the constraints on exonucleolytic nibbling? Are N nucleotides really added randomly? We have posed these questions in a variety of contexts chosen to permit a distinction between the various forces potentially capable of skewing the repertoire, e.g., the molecular events involved in TCR gene rearrangement, selection events during E-mediated clonal deletion in the thymus, and selection events predating MHC class I vs. class II restriction.

The extensive data we have accumulated provide a detailed picture of the actual $V_{\beta 17}^+$ TCR repertoire, and provide some hint of the forces that shape it, some previously unrecognized.

Materials and Methods

Mice. C57 Bl/6J (B6) and SJL/J (SJL) mice were obtained from Iffa-Credo (Les Oncins, France) and The Jackson Laboratory (Bar Harbor, ME).
large numbers of M13 clones emanating from different T cell populations were doubly screened for V~17a and V~17b. Single-stranded DNA was prepared from positively hybridized plaques and was sequenced by the dideoxy method using CAGTG-3' (VB17a) or the oligo 5'-ACAGAGCTACAGTG-3'. The cloned material was transfected into JM103 cells, and the resulting colonies were screened with either the oligo 5'-ACAGAGGTACACAGTG-3' from different T cell populations were doubly screened for V~17. The triple-labeled cells were sorted on a cytofluorimeter (ATC 3000; ODAM, Wissembourg, France) equipped with dual-laser excitation into CD4+ CD8+ V~17+, CD4+ CD8- V~17+, CD4- CD8+ V~17+, or CD4- CD8- V~17- populations of from 3 x 10^3 to 10^5 cells. 1 or 2 x 10^5 HeLa cells were added to each sample as carrier. RNA was prepared by the standard NP-40 lysis method (15) and was stored in water at ~20°C.

PCR Amplifications. cDNA was synthesized from 0.5-1 μg of RNA by the action of avian myeloblastosis virus reverse transcriptase in the buffer recommended by Cetus Corp. (Emeryville, CA) for PCR incubations. cDNA/RNA duplexes were denatured for 5 min at 95-100°C. After quick-cooling on ice, the mixture was supplemented with Taq DNA polymerase (1 U) and primers (50 pm), and the volume was brought up to 50 μl with PCR buffer (Cetus Corp.). For amplification of Vδ17 sequences, the primers were 5'-CTTTTGCTCCTGGG-3' in the V region and 5'-AGCACAGAGGTAGCCT-3' in the C region. 30 cycles of amplification were performed in a "DNA Thermal Cycler" (Perkin Elmer/Cetus, Emeryville, CA). Each cycle consisted of 30 s at 92°C, 30 s at 50°C, and 1 min at 72°C. The last cycle was followed by an incubation at 72°C for 10 min. A second round of amplification was performed starting with 1 μl of the first-round mixture. The PCR conditions were identical except that the V region primer was 5'-GTCCTGTGGCGCTGCAGGCTCTTTATGTTGCT-3' and the C region primer was 5'-GACAGAACCTTTGAAATCCTCTGCTTTTGATGG-3'. Both of these oligonucleotides contain artificial cloning sites (underlined): PstI in the former case, EcoRI in the latter.

Cloning and Sequencing. After the last amplification, the Taq polymerase was eliminated by phenol/chloroform then chloroform extraction, and the DNA was precipitated in ethanol. The pellet material was redissolved and digested with PstI and EcoRI and then digested M13mp19 DNA with PstI and EcoRI. The purified fragments were ligated with a vector prepared by digesting M13mp19 DNA with PstI and EcoRI. The ligated material was transfected into JM103 cells, and the resulting plaques were screened with either the oligo 5'-ACACAGGTACACAGTG-3' (V~17a) or the oligo 5'-ACACAGGTACACAGTG-3' (V~17b). Single-stranded DNA was prepared from positively hybridizing plaques and was sequenced by the dideoxy method using standard techniques.

Screening for Jα1.1. Large numbers of M13 clones emanating from different T cell populations were doubly screened for V~17 and Jα1.1. For the former, we used the PCR primer detailed above; for the latter, we used the oligo 5'-CTACAACGTGAGTGGTG-3'. Hybridization was at 45°C in 2x SSC, and washing was performed at 37°C in 2x SSC.

Contamination. Elaborate precautions were taken to prevent sample contamination, a major problem of PCR-based techniques. All solutions were aliquoted, and aliquots were used only once. Aside from the customary negative controls, a mock sample was processed along with each set of experimental samples: droplets of PBS without any cells were sorted and the entire procedure continued, including the screening of M13 plaques. This control ruled out contamination at any step along the way.

Results

Strategy

To evaluate how closely the actual T cell repertoire approximates the theoretical, we have chosen to focus on a single TCR Vδ region. This vastly reduces the number of sequences that must be generated to ensure statistical significance and greatly simplifies the sequence analysis. We have elected to concentrate on Vδ17 for a variety of reasons.

(a) Vδ17 is a particularly well-characterized TCR variable region, at both the gene and protein levels. cDNA and genomic sequences have been determined (14, 16, and K. Signorelli, unpublished results) and an effective anti-Vδ17 reagent is available (14).

(b) Vδ17 alleles are not always expressed as cell surface protein. A few inbred mouse strains (e.g., SJL) carry the expressed allele, Vδ17a1. Many others (e.g., B6) bear the nonexpressed counterpart, Vδ17b, which has a point mutation that specifies a stop codon, resulting in translation termination in the variable region (8). Thus, this allele is detectably transcribed into mRNA, but there is no corresponding protein at the cell surface. Hence, Vδ17b transcripts represent a virgin repertoire, incapable of being selected.

(c) Vδ17a+ T cells have been observed to undergo both negative (17) and positive (18-20) selection. For example, when clonal deletion of many, though not all, Vδ17a+ T cells occurs within the thymus of mice that express the E complex and an as yet uncharacterized ligand. By repeatedly backcrossing an Eα transgene (11) onto the SJL background, we have created a well-controlled system for studying this phenomenon: SJL vs. SJL.Eα, strains that should differ only by expression of the E complex.

Our strategy relies on the power of PCR technology. Thymus or lymph node cells are divided into discrete populations by sorting after staining with anti-CD4, -CD8, and -Vδ17 reagents. Vδ17 transcripts are amplified from the various pools by PCR using Cβ- and Vδ17-specific oligos. And finally, 50-100 transcripts are sequenced for each population. Although we have routinely tried to work with ~10,000 sorted cells, the technique is so sensitive that one can use <100.

Following this strategy, we have generated sequences from the following populations: thymus CD4+ CD8+ V~17b, thymus CD4+ CD8- V~17a, and CD4+ CD8- V~17a from E- mice, lymph node CD4+ V~17a, and CD8+ V~17b from E- mice; thymus CD4+ CD8+ V~17a and CD4+ CD8- V~17a from E- mice; and lymph node CD4+ V~17a and CD8+ V~17b from E+ mice. It should be kept in mind that each set of sequences routinely derives from three or more individual mice in order to ensure generality of the observations.

Nucleotide Sequences

Due to an editorial decision, we do not present nucleotide
Figure 1. Frequency of individual Jβ region usage. Jβ region frequencies were calculated from the data sets, and are shown here as histograms. (A) All Vβ17α sequences are compiled. (B) Individual histograms for each of the sorted thymic or lymph node populations from B6, SJL, or SJL.Eα mice.

sequences for the Vβ17+ TCRs from the nine cell populations. These can be obtained in raw form from the EMBL and GenBank data bases and in a considerably more useful annotated form directly from the authors. Several points emerge from our analysis of the data.

**J Region Usage.** A rearranged TCR β gene can use any one of 12 J segments, half associated with CB1 and the other half with CB2 (1, 2). Fig. 1 presents histograms of J region usage, both globally (A) and for individual sorted populations (B).
Considering the global histogram, one is immediately struck by the fact that J region usage is nonrandom. Jβ1.5 is used in only 0.9% of the TCRs, while Jβ2.6 is used 21.3% of the time. In general, there is a marked preference for Jβ2 over Jβ1: 80% vs. 20%. This skewing appears to be unrelated to thymic selection events because it occurs with both immature (thymic CD4+CD8-) and mature (peripheral CD4+ and CD8+) populations, with both E+ and E- mice, and even with the nonexpressed Va17b allele.

The histograms for the different cell populations, one does see some evidence for selection of particular Jβs. For example, Jβ1.1 is highly enriched during the transition from CD4+CD8+ to CD4+CD8- thymocytes in E- mice, increasing from 4.1% to 17.6%, and this heavy usage persists in peripheral CD4+ cells, at a level of 21.3%. Interestingly, enrichment for Jβ1.1 is not observed in the thymic CD4+CD8- or the peripheral CD4+ population of E- animals, nor in CD8 single-positives from either E+ or E- mice. To fortify the conclusion that T cells expressing Jβ1.1 undergo selection, we screened large numbers of M13 plaques derived from the different populations with both a Va17 and a Jβ1.1 probe. As is evident from Table 1, this experiment produced results in complete accord with the sequencing data: there is a clear difference in the number of Va17a+ clones that carry Jβ1.1 in the CD4+ population of E+ vs. E- mice, as well as in the CD4+ vs. CD8+ populations of both E+ and E- animals.

Jβ2.5 usage also shows evidence of selection. In E+ mice, passage from the CD4+CD8+ to the CD4+CD8- thymus compartment is accompanied by an increase in frequency from 16.2 to 24.7%. This is further augmented in the periphery where nearly one-third of CD4+ cells use this Jβ. In E- mice, there is no such increase in either thymic or peripheral CD4 single-positives.

Both of these variations in Jβ usage are statistically significant when assessed by the χ2 method (p < 0.05).

D Region Usage The TCR β locus has two D segments, one associated with Cβ1 and the other with Cβ2 (1, 2). In calculating the frequency of Dβ1 vs. Dβ2 usage, we have only taken into account those sequences for which an unambiguous assignment can be made: not always possible given the marked sequence homology between the two segments.

### Table 1. Jβ1.1 Usage in Va17+ T Cells

| Mouse strain | CD4    | CD8    |
|--------------|--------|--------|
| E negative   | 165/1,232 (13.4%) | 43/1,024 (4.1%) |
| E positive   | 1/972 (0.1%) | 66/1,140 (5.7%) |

Values represent the numbers (and percentages) of M13 plaques that were positive after hybridization to a specific Jβ1.1 probe (see Materials and Methods for details). Plaques are derived from lymph node CD4+ or CD8+ T cells from SJL or SJL.Ec.

Coupled with the fact that extensive exonuclease digestion can occur during the rearrangement process, Dβ1 is preferred over Dβ2 both globally (54% vs. 46%) and in the unselectable Va17b cell population (62% vs. 37%). Skewing in the individual T cell populations is variable, with no obvious logic.

Dβ1 is found in combination with Jβ2 about twice as often as with Jβ1. This is true for all T cell populations.

P Nucleotide Addition. If no exonucleolytic nibbling has taken place, one or two P nucleotides (3) may be found at each of the four recombined segment termini: the 3’ end of the V region, the 5’ and 3’ ends of the D segments, and the 5’ end of the J segment.

Indeed, we find P nucleotides in a number of sequences. Interestingly, they are found more often at VD joints (at 67% of undigested 3’V or 5’D termini) then at DJ joints (45% of undigested 3’D or 5’J termini).

Exonucleolytic Chewing. Rearrangement of TCR and Ig genes often entails a variable degree of exonucleolytic nibbling of the recombining termini. Table 2 lists the number of nucleotides removed from the 3’V and 5’J termini (a similar analysis for the D termini would not be meaningful because the deletion assignments are often ambiguous).

At the 3’V end, the vast majority of sequences show removal of zero to four bases, but in a few cases the nibbling does extend further, up to 11 bases. A very similar profile is observed with the unexpressed Va17b sequences. At the 5’J end, removal of zero to four bases is also standard, but there are some surprising differences with the different J segments.

### Table 2. Exonucleolytic Nibbling during Vβ17 TCR Rearrangement

| No. of bases removed | Vβ17a | Vβ17b | Jβ2.1 | Jβ2.3 | Jβ2.6 |
|----------------------|-------|-------|-------|-------|-------|
| 0                    | 13.6  | 11.5  | 8.8(1)* | 46.0  | 19.7(3) |
| 1                    | 14.6  | 9.6   | 23.5(2) | 11.8(1) | 5.3(0) |
| 2                    | 15.0  | 17.3  | 8.8    | 14.4  | 3.8(2) |
| 3                    | 17.9  | 15.3  | 10.8(1) | 9.2(1) | 12.1(5) |
| 4                    | 20.7  | 19.2  | 11.8(2) | 5.2(1) | 19.7(7) |
| 5                    | 5.3   | 7.6   | 11.8   | 6.5   | 13.6(1) |
| 6                    | 8.6   | 13.4  | 11.8   | 3.9   | 12.8(0) |
| 7                    | 1.8   | 1.9   | 5.9    | 1.3   | 11.4(2) |
| 8                    | 1.4   | 3.8   | 2.9    | 0     | 0.7(0) |
| 9                    | 0.3   | 0     | 2.9    | 1.3   | 0.7(0) |
| 10                   | 0     | 0     | 0      | 0     | 0(0)   |
| 11                   | 0.3   | 1.0   | 0      | 0     | 0(0)   |

n = 279 n = 52 n = 102 n = 76 n = 132

* The values in parentheses indicate the corresponding number of occurrences in Vβ17b sequences.

992 The Actual Versus the Theoretical Repertoire of Vγ17+ T Cells
For Jβ2.3, almost half of the sequences show no deletion. For Jβ2.6, the distribution seems bimodal: several sequences are undeleted, but the majority have three to six bases removed. The Jβ2.1 pattern seems different again. While the number of Vβ17b sequences is too small for a rigorous comparison, one notes that the Vβ17b percentages for Jβ2.6 seem to parallel the Vβ17a values. Thus, the peculiar distribution that we observe directly reflects molecular recombination events rather than selective influences, and exonucleolytic nibbling seems a fairly controlled process, sensitive to local DNA structure.

**N Nucleotide Addition.** A variable number of N nucleotides can be inserted at the VD and DJ junctions. For the Vβ17+ TCRs analyzed here, the number added per sequence ranges from 0 to 14, reaching a maximum of 11 for a single junction. The average number of N nucleotides per sequence shows little variation in the different T cell populations, hovering between 2.2 and 3.4, with an average of 2.8.

As a further refinement, we sought to determine whether N region addition shows any nucleotide preferences. Table 3 lists the frequency with which each of the four bases is used at each junction, globally and in individual cell populations. Interestingly, base utilization at the VD and DJ junctions is not identical. The nucleotides inserted between V and D are rich in cytosine, while those added between D and J show no such enrichment: 38.9-57.9% vs. 10.6-23.3%.

**Protein Sequences**

Protein sequences for the Vβ17+ TCRs from the nine cell populations are presented in Fig. 2. Only the junctional regions are shown. Several points emerge as salient.

**CDR3 Lengths.** By analogy with Ig heavy chains, the CDR3 of a TCR β chain should extend from the serine situated at position 94 to the phenylalanine in the motif FGXG, conserved in almost all TCR J regions (21). Fig. 3 shows histograms of CDR3 lengths, again globally (A) and for the individual sorted populations (B).

The overall average length is 10.0 amino acids, ranging from 6 to 15. The profiles from E- mice exhibit very little variability, while those from E+ animals do show one difference worth mentioning: the CDR3s from peripheral CD8+ cells are noticeably longer than those from peripheral CD4+ cells (10.7 amino acids on average, vs. 9.3).

**NDN Amino Acids.** The impressive variability of the TCR β chain CDR3 regions rests partly on N addition and partly on the D segment choice and its "processing". The particular residues that one finds in the NDN stretch are heavily influenced by the coding potential of the D segments. The amino acids that can be specified by Dβ1 and Dβ2 are indicated in Figure 4. It is worth keeping in mind that all three reading frames can be used and that glycine predominates in each.

Table 4 illustrates amino acid usage in the NDN region of our Vβ17+ TCRs. Clearly, the patterns are nonrandom. Considering first those amino acids encoded within Dβ, we can make two points of interest. First, as expected, glycine is by far the most frequently used residue (fully one third of all the NDN amino acids are glycine). Second, one residue appears to be differentially used in the various cell populations (tryptophan is used distinctly more frequently in peripheral CD8+ cells than in CD4+ cells). Considering those amino acids not encoded within Dβ, we can again bring out two points. First, cysteine is almost never found, perhaps not surprisingly considering its potential to interfere with the Ig fold. Second, proline is frequently observed, but this can probably be explained by the fact that its codon is CCN.

As discussed above, N nucleotides inserted at the VD junction are enriched in Cs; in addition, P nucleotides are frequently detected at the 5′D terminus, where they occur as either one or two Cs.

### Table 3. Base Distribution in N Nucleotides

|          | G    | A    | T    | C    |
|----------|------|------|------|------|
| Vβ17b,CD4+*8- | 19.6 | 17.0 | 15.6 | 48.1 |
| Thy CD4+*8-   | 24.3 | 9.7  | 11.3 | 54.3 |
| Thy CD4+*8-   | 20.6 | 18.6 | 19.6 | 43.3 |
| LN CD4+      | 8.0  | 19.3 | 14.8 | 57.9 |
| LN CD8+      | 14.0 | 19.3 | 18.3 | 48.4 |
| Thy CD4+*8-   | 16.4 | 17.9 | 17.9 | 47.8 |
| Thy CD4+*8-   | 21.9 | 21.9 | 17.2 | 39.0 |
| LN CD4+      | 22.9 | 14.6 | 9.4  | 53.1 |
| LN CD8+      | 21.5 | 16.5 | 23.1 | 38.9 |

|          | G    | A    | T    | C    |
|----------|------|------|------|------|
| All sequences | 31.2 | 28.1 | 23.8 | 16.7 |
| B6       | 23.0 | 33.8 | 21.6 | 21.6 |
| SJL      | 25.0 | 28.3 | 23.3 | 23.3 |
| SJL.Ea   | 26.9 | 27.9 | 34.6 | 10.6 |
| SJL.Ea   | 31.7 | 28.7 | 24.8 | 14.8 |
| SJL      | 37.1 | 26.7 | 19.0 | 17.2 |
| SJL      | 30.9 | 35.5 | 19.1 | 14.5 |
| SJL      | 44.2 | 27.4 | 15.8 | 12.6 |
| SJL      | 32.5 | 27.7 | 19.3 | 20.5 |
| SJL      | 30.0 | 17.7 | 36.9 | 15.4 |
| B6(Vβ17b) | THYMIC 4+8+ | THYMIC 4+ | THYMIC 4+ | LYMPH NODE 4+ | LYMPH NODE 8+ |
|-----------|-------------|-----------|-----------|--------------|--------------|
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |
| ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG | ASSLPGDRKSLFSQG |

**Figure 2.** Protein sequences. Amino acid sequences are deduced from the nucleotide sequences and displayed in the standard one-letter code. Bold letters indicate amino acids specified by the N, D, and N bases; normal characters correspond to V- or J-encoded amino acids. The nucleotide sequences from which these sequences are derived have been given accession numbers X61756-X61764 in the EMBL/GenBank Data Libraries.
Figure 3. Lengths of the CDR3 loops. Each histogram plots the number of sequences vs. the size of the CDR3 loops, deduced from the data sets. The definition of the CDR3 loop is based on the homology of TCRβ chains with Igs (21-23, 35). Following reference 21, CDR3 starts downstream from the conserved Ser at position 94, and ends immediately upstream from the conserved Phe at position 108 (numbering according to reference 21); thus, . . . CAS<->CDR3<->FGXG . . .

In Table 5, we have grouped amino acids according to the frequency at which they are encoded in the D segments of Vβ17+ TCRs, and have weighed each one's occurrence according to the number of codons that specify it. Proline, glutamic acid, tryptophan, and aspartic acid seem to be selected for; conversely, isoleucine, tyrosine, methionine, cysteine, leucine, and alanine appear to be selected against. In general, there is a preference for charged and polar residues over hydrophobics.

Discussion

General Properties of Vβ17+ TCRs

We have sequenced >600 transcripts that specify TCRs carrying the Vβ17 variable region. From this large data base, certain conclusions can be drawn about the "typical" junctional region of Vβ17+ TCRs. Some of the conclusions are particularly interesting in light of past observations on Ig receptors.

Jβ Usage. Vβ17+ TCRs do not randomly use joining segments. Perhaps most striking is the finding that Jβ2s are used about four times as often as Jβ1s. This preference is evident with both expressed and nonexpressed transcripts, indicating that it is independent of repertoire selection events. One explanation may lie in the structure of the individual recombination signals: all of the heptamer and nonamer signals 5' of the Jβ1 segments are separated by 13 nucleotides (22), while those 5' of the Jβ2s are all 12 nucleotides apart (23). This difference, though small, may render the Jβ1 segments less recognizable by the recombination machinery. In fact, it has already been shown that just such a single nucleotide difference can measurably reduce the ability of an artificial substrate to undergo recombination in pre-B cells (24).

A preference for Jβ2s was foretold by previous reports that, no matter which Vβ segment they carry, TCR cDNAs bearing Jβ2 fragments are isolated more frequently from recombinant libraries (25), and TCR genes with rearranged Jβ2 fragments are detected more frequently on Southern blots (26). Intriguingly, skewed Jβ usage was not observed in fetal T lymphocytes (27), but we have seen it in neonatal T cells (27a).

More novel is the observation that the Jβ2s, themselves, are subject to uneven usage: Jβ2.1, Jβ2.5, and Jβ2.6 being particularly favored. Again, this nonrandom use of joining
Table 4. Amino Acid Frequency in NDN-encoded Stretches

|    | G | A | S | T | L | I | V | M | F | Y | W | H | R | K | D | E | Q | N | C | P |
|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | 1 | 1 | 4 | 9 | 6 | 5 | 2 | 7 | 0 | 2 | 7 | 0 | 1 | 1 | 0 | 4 | 9 | 2 | 7 | 1 |
| 32 | 1 | 1 | 4 | 9 | 6 | 5 | 2 | 7 | 0 | 1 | 1 | 0 | 4 | 9 | 2 | 7 | 1 | 1 | 1 | 1 |
| 33 | 1 | 1 | 4 | 9 | 6 | 5 | 2 | 7 | 0 | 1 | 1 | 0 | 4 | 9 | 2 | 7 | 1 | 1 | 1 | 1 |

31.3 2.7 4.9 6.5 2.7 0 2.7 0 1.1 0 4.9 2.7 1.9 1.1 3.2 3.8 4.3 1.1 1.1 1.1 14.0 V\textsubscript{a}17\textsubscript{b} B6

31.6 2.9 4.0 8.6 3.4 0 2.9 0 1.7 1.1 4.0 1.7 12.6 1.1 12.1 2.3 1.7 0.6 0.6

32.1 3.4 4.3 7.7 3.4 0.8 2.5 0.8 3.0 0.4 2.1 2.1 11.1 1.7 7.2 2.6 4.3 2.1 0 0.7 7.7 Thymic \textsuperscript{4+8-} SJK

32.8 3.4 5.2 9.1 2.8 2.4 1.0 0.7 2.4 1.0 1.4 2.1 7.7 2.1 8.0 2.4 4.5 1.0 1.3 9.1 Lymph node \textsuperscript{4+8-} SJK

30.1 5.1 4.7 7.6 6.2 1.1 3.3 0 0.7 8.3 1.1 6.9 0.7 8.3 3.6 2.9 2.2 0 0.6 7.2 Lymph node \textsuperscript{4+8-} SJK

32.3 2.9 3.6 8.0 2.9 1.8 2.5 0 1.5 0.3 2.9 1.5 10.2 1.8 8.7 2.5 5.8 1.5 0 9.1 Thymic \textsuperscript{4+8-} SJK

36.8 4.0 10.7 2.7 0.4 2.2 1.3 1.3 0.4 4.4 0.8 7.5 0.8 8.8 2.7 4.0 0.8 0.8 4.9 Thymic \textsuperscript{4+8-} SJK

32.5 3.6 2.9 9.0 6.1 4.0 0 1.4 0 0.5 0 1.4 0.9 9.0 1.4 12.3 3.3 3.3 0.5 0 10.8 Lymph node \textsuperscript{4+8-} SJK

28.8 4.2 4.0 5.7 5.4 0.8 2.5 0.3 2.5 0.6 5.3 0.6 8.5 0.6 9.3 2.8 1.4 0.3 0 5.9 Lymph node \textsuperscript{4+8-} SJK

36.8 4.0 10.7 2.7 0.4 2.2 1.3 1.3 0.4 4.4 0.8 7.5 0.8 8.8 2.7 4.0 0.8 0.8 4.9 Thymic \textsuperscript{4+8-} SJK

32.5 3.6 2.9 9.0 6.1 4.0 0 1.4 0 0.5 0 1.4 0.9 9.0 1.4 12.3 3.3 3.3 0.5 0 10.8 Lymph node \textsuperscript{4+8-} SJK

28.8 4.2 4.0 5.7 5.4 0.8 2.5 0.3 2.5 0.6 5.3 0.6 8.5 0.6 9.3 2.8 1.4 0.3 0 5.9 Lymph node \textsuperscript{4+8-} SJK

Table 5. Amino Acid Usage in the NDN Stretch

| No. of occurrences | No. of occurrences in stretch (all \textsubscript{Va}17\textsubscript{a} sequences) | Weighed no. of occurrences |
|--------------------|---------------------------------------------|-----------------------------|
| 0                  | P  176                                       | 44                          |
|                    | S   98                                       | 16                          |
|                    | E   64                                       | 32                          |
|                    | V   52                                       | 13                          |
|                    | F   40                                       | 20                          |
|                    | H   32                                       | 16                          |
|                    | K   28                                       | 14                          |
|                    | N   25                                       | 12                          |
|                    | I   21                                       | 7                           |
|                    | Y   10                                       | 5                           |
|                    | M   8                                        | 8                           |
|                    | C   2                                        | 1                           |
| 1                  | R   205                                      | 34                          |
|                    | L   90                                       | 15                          |
|                    | W   88                                       | 88                          |
|                    | Q   79                                       | 39                          |
| 2                  | D   193                                      | 96                          |
|                    | T   177                                      | 44                          |
|                    | A   82                                       | 20                          |
| 14                 | G   708                                      | 118                         |

An extensive study of human Ig heavy chain CDR3 regions has recently been published (27). Over 100 sequences were produced from the PBL of six individuals, making no selection for particular heavy chains. The six possible J segments were also used distinctly nonrandomly in these receptors, JH4 being found in over half the sequences and JH1 and JH2 in <2%.

N Nucleotide Addition. On average, \textsubscript{Va}17\textsuperscript{+} TCRs carry three N nucleotides per sequence; they may have as few as zero or as many as 14. The likelihood of finding N nucleotides is the same at the VD and DJ junctions, but the base composition at each seems to differ. There is an elevated GC content at the VD joint (with a marked enrichment in Cs), but the four bases occur more or less equally at the DJ joint.

Human Ig heavy chains show a somewhat different profile of N additions (e.g., 27; and Fig. 5 A). First of all, more Ns are added per sequence: an average of 6.2 and a maximum of 50. Second, at both the VD and DJ junctions the N additions are GC rich, but G and C are represented roughly equally. These variations can not be attributed to particularities of the \textsubscript{Va}17\textsuperscript{+} TCR or of the human IgH data bases because a smaller set of \textsubscript{Va}17\textsuperscript{-} TCR (28) and murine IgH (29) sequences show similar differences.

Since the distinct profile of N nucleotide additions is characteristic of \textsubscript{Va}17\textsubscript{a} TCRs as a whole and of unselectable \textsubscript{Va}17\textsubscript{b} TCRs, it is difficult for one to invoke a contribution from repertoire selection events. Rather, some feature of the recombination machinery must be responsible. It is not known with certainty what enzyme(s) is (are) responsible for the addition of N nucleotides to either TCR or Ig gene segments. Nevertheless, several facts point to terminal deoxynucleotidyl transferase (TdT). For example, this is the only enzyme presently known to be capable of template-independent nucleotide additions (30). In addition, there seems to be a good correlation between the cell subsets that express TdT and those that support rearrangement events, including N nucleotide

1 Abbreviation used in this paper: TdT, terminal deoxynucleotidyl transferase.

14 G 708 118

996 The Actual Versus the Theoretical Repertoire of \textsubscript{Va}17\textsuperscript{+} T Cells
addition (31, 32). Finally, when an expressible TdT cDNA was introduced into a pre-B cell line, the frequency of N nucleotides added to a plasmid substrate increased, though not to the anticipated level (33). According to Alt and Baltimore (34), TdT prefers to polymerize dGTP. This would be consistent with the elevated GC content of N additions at the TCR VD junction and both IgH junctions. Given the contribution of Cs to this over-representation at the TCR VD junction, one is led to suggest that, in this case, N nucleotides are polymerized from the 3' end of the antisense strand of D rather than from the 5' end of the sense strand of V. Why this asymmetry is not observed at the IgH junction is mysterious and, together with the observation that the TCR DJ junction is not GC rich at all, prompts one to hypothesize that, in this case, N nucleotides are polymerized from the 3' end of the antisense strand of D rather than from the 5' end of the sense strand of V. Why this asymmetry is not observed at the IgH junction is mysterious and, together with the observation that the TCR DJ junction is not GC rich at all, prompts one to hypothesize that, in this case, N nucleotides are polymerized from the 3' end of the antisense strand of D rather than from the 5' end of the sense strand of V.

CDR3 Lengths and Structure. V$_{\beta}$17 + TCRs have a rather narrow distribution of CDR3 lengths: average, 10; range, 6–15. As illustrated in Fig. 5 B, this distribution is much tighter than what was observed with IgH chains: long CDR3 loops (>13 amino acids), which are very common in IgH, are virtually absent in V$_{\beta}$17 + TCRs. These differences follow from their distinct profiles of N nucleotide addition, but also depend on the relative degree of exonuclease nibbling. The J segments actually contribute limited variability, because of their homology and skewed usage. The narrower histogram of TCR CDR3 lengths seems logical given that TCRs are obliged to interact with MHC molecules carrying peptide antigens, while IgH interact with antigens as diverse as small hapten and large globular proteins. Thus, one is tempted to suggest an active size selection, either during differentiation or throughout evolution. The former possibility seems unlikely, however, because V$_{\beta}$17b + TCRs also have a tight distribution of CDR3 lengths.

Perhaps reflecting this restrained variability, the V$_{\beta}$17 + TCRs show a fairly well conserved structural feature in the CDR3s. If one plots the CDR3 amino acids onto the loop structure suggested by Chothia et al. (35), one sees that one side of the loop consists of amino acids contributed by the V and the NDN stretches, while the other is composed, in the vast majority of sequences, of amino acids encoded by the J segment (Fig. 6). Consequently, one side of the loop (and in particular, positions 3, 4, and 5) would seem to be much more variable than the other; perhaps the two sides interact with distinct components of the MHC molecule/peptide antigen duplex. Not surprisingly, this feature is not evident in the CDR3s of IgH chains.
Selection of Vβ17 TCRs

The sequences that we have generated derive from nine different sorted populations. Three of them can be considered unselected: the C57Bl/6 cells because Vβ17b transcripts do not give rise to cell surface protein, and the thymus CD4 + CD8 + cells from E − and E + mice because they have low levels of TCR, (and were sorted as such) and are generally considered to be an immature, pre-selection subset. The other six populations have undergone selection of various types and allow us to view the effects of selection forces on the repertoire.

E − and E + Mice. Almost all T cells carrying Vβ17a + TCRs are capable of responding to the E complex plus an unidentified B cell ligand (14). To avoid autoreactive cells, E + mice eliminate Vβ17 + T cells from their repertoire (17). But this is never complete, so that when one compares E − with E + mice, one sees a reduction from 8-10% Vβ17 + T cells in the peripheral CD4 + compartment to 2%. A question that has interested us is whether the remaining cells have distinctive junctional region features.

Of all the parameters analyzed, only J region usage consistently shows variability between E − and E + mice. Two Jβs are involved. Jβ1.1 is highly enriched in the Vβ17 + CD4 single-positive cells of E − mice, suggesting a positive selection event mediated by one of the SJL MHC molecules, probably A1 since it is the CD4 + , not the CD8 + , cells that are involved. An increased level of Jβ1.1 is not seen in the corresponding cells of E + mice, implying that cells expressing this Jβ undergo efficient E-mediated negative selection. Jβ2.5, on the other hand, is highly enriched in the Vβ17 + CD4 single-positive cells of E − , but not E + , mice. Fully one-third of the peripheral CD4 + 's express this J segment. This result suggests that cells expressing Jβ2.5 are preferentially positively selected by the E molecule, are more resistant to negative selection, or both.

Such influences by MHC-mediated selection events were not really expected. The dogma has been that particular VBs are negatively selected irrespectively of α chain and junctional region contributions. This assertion is consistent with the mapping of TCR residues involved in negative selection mediated by MHC molecules in conjunction with various superantigens (16, 38, 39) but is not supported by the observation that only partial intrathymic clonal deletion is observed in mls-positive transgenic mice that express only the β chain from an mls-reactive clone (40, 41, and K. Signorelli, unpublished results).

CD4 + and CD8 + Cells. T cells expressing CD4 and CD8 are selected by different MHC molecules in the thymus (class II vs. class I) and recognize foreign antigens in the context of different MHC molecules on APC (again, class II vs. class I). Robey et al. (42) have recently reported that ectopic expression of CD4 on peripheral CD8 + lymphocytes enables at least some of them to react allelogeneically across an MHC class II difference, suggesting that some TCRs selected on class I molecules have the potential to interact with class II molecules. Nonetheless, we have questioned whether junctional region sequences somehow predetermine the class I/class II restriction dichotomy.

Some dissimilarities do emerge from a comparison of the Vβ17 + TCRs of peripheral CD4 + and CD8 + cells. (a) Although, in general, CDR3 lengths show a relatively tight distribution, the greatest variation was observed between lymph node CD4 + and CD8 + cells (9.3 vs. 10.7 residues on average). Long CDR3 loops (>12) are significantly more frequent in CD8 than in CD4 cells (27% vs. 13% of sequences). (b) Interesting differences become apparent when we plot the frequency of individual amino acids or of amino acid groups at each position in the CDR3 loop (Fig. 7). Positions 1 and 2 are dominated by leucine and serine residues contributed by the 3' end of the Vβ17 segment; proline (contributed by P or N nucleotides) is also quite frequent at position 2. Positions 3, 4, and 5 (as noted above) are far more variable. The most striking difference between sequence sets is the frequent presence of tryptophan in the CDR3s of CD8 + cells and its virtual absence in those of CD4 + cells. Close to one-third of the class I-restricted TCRs carry a tryptophan at positions 3, 4, or 5 of the CDR3. This observation should be considered in light of the nature of Trp, the bulkiest of amino acids, usually found buried in hydrophobic cores. Other differences are more subtle. Polar amino acids and glycine are quite frequent in the CDR3s of CD4 + cells; charged residues are preferentially acidic at all positions. Polar amino acids and glycine are less prevalent in the CDR3s of CD8 + cells, particularly at position 3. The distribution of charged amino acids at individual positions in CDR3. Each horizontal bar depicts the frequency of amino acids or classes thereof at each of the first five positions of CDR3, within sequences derived from CD4 + or CD8 + lymph node cells. For greater representativity, we pooled data from SJL and SJL/Esr mice (for CD4; n = 113, for CD8, n = 140). The differences we observe between sequences from CD4 + or CD8 + cells at positions 3 and 4 are statistically significant ($\chi^2$, p < 0.01).
acids shows a distinct profile: while positive or negative charges are equally represented at positions 3 and 5, there is a very strong bias for negative charges at position 4.

At present, we can only speculate whether these differences between class I- and class II-restricted TCRs will prove general, and not solely restricted to Vβ17+ β chains. A preliminary search of the literature suggested that the differential use of Trp might indeed be general (although there were some exceptions, such as in reference 43). We do not know whether any of the differences are related to requirements for contacts with class I vs. class II molecules, or with potentially different types of peptides presented by these molecules.

**Implications**
The repertoire of Vβ17+ T cells actually used in the peripheral immune system is much smaller than the theoretical repertoire calculated by assuming entirely random operation of the recombination machinery and random export of mature cells from the thymus. J region usage is skewed, as is D region usage. The appearance of P nucleotides is nonrandom, as is the addition of N nucleotides. The overall length of the VDJ junctional region seems rather tightly controlled. We assume that these observations are not peculiar to Vβ17+ TCRs, and actually have preliminary evidence to support this assumption from Vβ6 junctional region sequences (U. Hartwig and M. Bogue, unpublished results). Estimates of repertoire size may need to be adjusted accordingly.

We thank P. Bohn, P. Gerber, and S. Vicaire for technical assistance, and A. Staub and F. Rufvenach for oligonucleotide synthesis.

This work was supported by institutional funds from the INSERM and CNRS, and by grants to D. Mathis and C. Benoist from the National Institutes of Health and the Association pour la Recherche sur le Cancer. S. Candeias received fellowships from the Ministère de la Recherche et de la Technologie, the Association pour la Recherche sur le Cancer, and the Université Louis Pasteur.

Address correspondence to Diane Mathis, Laboratoire de Génétique Moléculaire des Eucaryotes, Unité 184 de Biologie Moléculaire et de Génie Génétique de INSERM, Institut de Chimie Biologique, 11, rue Humann, 67085 Strasbourg, France. S. Candeias' present address is the National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

Received for publication 13 May 1991 and in revised form 22 July 1991.

**References**

1. Davis, M. 1988. T cell receptor genes. In Molecular Immunology. B. Hames, and D. Glover, editors. IRL Press, Oxford. 61–79.

2. Wilson, R., E. Lai, P. Concannon, R. Barth, and L. Hood. 1988. Structure, organization and polymorphism of murine and human T-cell receptor α and β gene families. *ImmunoL Rev*. 101:149.

3. Lafaille, J., A. DeCloux, M. Bonneville, Y. Takagaki, and S. Tonegawa. 1989. Junctional sequences of T cell receptor βγ genes: implication for T cell lineages and for a novel intermediate of V(D)-J joining. *Cell*. 59:859.

4. Davis, M., and P. Bjorkman. 1988. T-cell antigen receptor genes and T-cell recognition. *Nature (Lond.)*. 334:395.

5. Behlke, M., H. Chou, K. Hoppi, and D. Loh. 1986. Murine T cell receptor mutants with deletions of β-chain variable region genes. *Proc. Natl. Acad. Sci. USA*. 83:767.

6. Haqqi, T.M., S. Banerjee, G. Anderson, and C.S. David. 1989. RIII S/J (H-2j). An inbred mouse strain with a massive deletion of T cell receptor Vβ genes. *J. Exp. Med.* 169:1903.

7. Kotzin, B.L., V. Barr, and E. Palmer. 1985. A large deletion within the T-cell receptor β-chain gene complex in NZW mice. *Science (Wash. DC)*. 229:167.

8. Wade, T., J. Bill, P. Marrack, E. Palmer, and J.W. Kappler. 1988. Molecular basis for the nonexpression of Vβ17 in some strains of mice. *J. Immunol*. 141:2165.

9. MacDonald, H.R., A.L. Glasebrook, R. Schneider, R.K. Lees, H. Pircher, T. Pedrazzini, O. Kanagawa, J.-F. Nicolas, R.C. Howe, R.M. Zinkernagel, and H. Engelhard. 1989. T-cell reactivity and tolerance to Mls' encoded antigens. *ImmunoL Rev*. 107:89.

10. Blackman, M., J. Kappler, and P. Marrack. 1990. The role of the T cell receptor in positive and negative selection of developing T cells. *Science (Wash. DC)*. 248:1335.

11. Le Meur, M., P. Gerlinger, C. Benoist, and D. Mathis. 1985. Correcting an immune response deficiency by creating Ecr gene transgenic mice. *Nature (Lond.)*. 316:36.

12. van Ewijk, W., Y. Ron, J. Monaco, J. Kappler, P. Marrack, M. Le Meur, P. Gerlinger, B. Durand, C. Benoist, and D. Mathis. 1988. Compartimentalization of MHC class II gene expression in transgenic mice. *Cell*. 53:357.

13. Benoist, C., and D. Mathis. 1989. Positive selection of the T cell repertoire: when and where does it occur? *Cell*. 58:1027.

14. Kappler, J., T. Wade, J. White, E. Kushner, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987a. A T cell receptor Vβ segment that imparts reactivity to a class II major histocompatibility complex protein product. *Cell*. 49:263.

15. Glasebrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Labora-
16. Cazenave, P.-A., P.N. Marche, E. Jouvin-Marche, D. Voegtlé, F. Bonhomme, A. Bandeira, and A. Coutinho. 1990. Vβ17 gene polymorphism in wild-derived mouse strains: two amino acid substitutions in the Vβ17 region greatly alter T cell receptor specificity. *Cell.* 63:717.

17. Kappler, J., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. *Cell.* 49:273.

18. Zuniga-Pflucker, J.C., D.L. Longo, and A.M. Kruisbeek. 1989. *J.* Exp. Med. 173:395.

19. Iwabuchi, K., I. Negishi, H. Arase, C. Iwabuchi, K. Ogasawara, R.A. Good, and K. Ono. Positive selection of CD4-CD8+ T cells in the thymus of normal mice. *Nature (London)*. 338:76.

20. Iwabuchi, K., I. Negishi, H. Arase, C. Iwabuchi, K. Ogasawara, R.A. Good, and K. Ono. Positive selection of CD4-CD8+ T cells in the thymus of normal mice. *Nature (London)*. 338:76.

21. Kabat, E., T. Wu, M. Reid-Miller, H. Perry, and K. Gottesman. 1989. Influence by clonal elimination in the thymus. *Cell.* 49:273.

22. Blackman, M.A., P. Marrack, and J. Kappler. 1989. Influence of the major histocompatibility complex on positive thymic selection of Vβ17+ T cells. *Science* (Wash. DC). 244:214.

23. Kabat, E., T. Wu, M. Reid-Miller, H. Perry, and K. Gottesman. 1989. Sequences of Immunological Interest, 4th edition. Department of Health and Human Services, Bethesda, MD.

24. Gascoigne, N., Y. Chien, D. Becker, J. Kavalier, and M. Davis. 1984. Genomic organization and sequence of T-cell receptor B-chain constant- and joining-region genes. *Nature (London)*. 310:387.

25. Feeney, A. 1990. Lack of N regions in fetal and neonatal mouse immunoglobulin V-D-J junctional sequences. *J.* Exp. Med. 172:1377.

26. Bollum, F. 1974. Terminal deoxynucleotidyl transferase. In *The Enzymes.* Vol. X. P. Boyer, editor. Academic Press, New York. 145–171.

27. Desideroio, S., G. Yancopoulos, M. Paskind, E. Thomas, M. Boss, N. Landau, F. Alt, and D. Baltimore. 1984. Insertion of N regions into heavy chain genes correlated with expression of terminal deoxynucleotidyl transferase in B cells. *Nature (London)*. 311:752.

28. Yancopoulos, G.D., T.K. Blackwell, H. Suh, L. Hood, and F.W. Alt. 1986. Introduced T cell receptor variable region gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. *Cell.* 44:251.

29. Landau, N.R., D.G. Schatz, M. Rosa, and D. Baltimore. 1987. Increased frequency of N-region insertion in a murine pre-B-cell line infected with a terminal deoxynucleotidyl transferase retroviral expression vector. *Mol. Cell. Biol.* 7:3237.

30. Alt, F., and D. Baltimore. 1982. Joining of immunoglobulin heavy chain gene segments: implications from a chromosome with evidence of three D-Jf fusions. *Proc. Natl. Acad. Sci. USA.* 79:4148.

31. Choithia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of the T-cell αβ receptor. *EMBO (Eur. Mol. Biol. Organ.)* J. 7:3745.

32. Nikoliz-Zugic, J. 1991. Phenotypic and functional stages in the intrathymic development of αβ T cells. *Immunol. Today.* 12:65.

33. Boyd, R.L., and P. Hugo. 1991. Towards an integrated view of thymopoiesis. *Immunol. Today.* 12:71.

34. Pullen, A.M., T. Wade, P. Marrack, and J. Kappler. 1990. Identification of the region of T cell receptor β chain that interacts with the self-superantigen Mls-1a. *Cell.* 61:1365.

35. Choi, Y., A. Herman, D. DiGiusto, T. Wade, P. Marrack, and J. Kappler. 1990. Residues of the variable region of the T-cell receptor β-chain that interact with S. Aureus toxin superantigens. *Nature (London).* 346:471.

36. Blackman, M.A., H. Gerhard-Brugert, D.L. Woodland, E. Palmer, J.W. Kappler, and P. Marrack. 1990. A role for clonal inactivation in T cell tolerance to Mls-1a. *Nature (London).* 345:540.

37. Pircher, H., F. Ramsdell, J. Elliott, D. Raulet, D. Kioussis, R. Axel, and B.J. Fowlkes. 1991. Expression of CD4 in transgenic mice. *Nature (London).* 345:540.

38. Dasaka, J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and R. deze. 1991. Expression of CD4 in transgenic mice alters the specificity of CD8 cells from allogeneic major histocompatibility complex. *Proc. Natl. Acad. Sci. USA.* 88:608.

39. Roche, E., F. Ramsdell, J. Elliott, D. Raulet, D. Kioussis, R. Axel, and B.J. Fowlkes. 1991. Expression of CD4 in transgenic mice alters the specificity of CD8 cells from allogeneic major histocompatibility complex. *Proc. Natl. Acad. Sci. USA.* 88:608.

40. Danska, J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and C.G. Fathman. 1990. The presumptive CDR3 regions of both T cell receptor α and β chains determine T cell specificity for myoglobin peptides. *J.* Exp. Med. 172:27.