Heterodimeric TCR-\(\alpha/\beta\)s are made up of combinations of V, D, J, and C elements. The majority of laboratory inbred mouse strains are of the V(36) haplotype and have at least 20 V(3) genes from which to construct TCRs (1, 2); however, a number of strains have been reported to have deletions of large portions of the V(3) locus on chromosome 6 (3-5), and these mice must survive with a considerably reduced potential TCR repertoire.

Generally, all the variable elements of the TCR (V\(\alpha\), J\(\alpha\), V\(\beta\), D\(\beta\), and J\(\beta\)) contribute to binding of a conventional antigen-MHC complex. A second group of antigens that stimulate T cells via their TCR V\(\beta\) element alone, essentially with no regard for the other components of the receptor, has recently been documented and termed superantigens (6, 7).

The self superantigens, which include the much studied but little understood mixed lymphocyte stimulating locus (Mls)\(^1\) determinants, have been shown to play an important role in shaping the T cell repertoire. T cells reactive with such self superantigens are eliminated in the thymus by clonal deletion (8), the mechanism of which is as yet unknown. CBA/J and CBA/CaJ are closely related mice, for example, and yet they differ by \(\sim 30\%\) of their T cell repertoire because the expression of Mls-1\(^a\) and Mls-2\(^a\) or -3\(^a\) in CBA/J animals leads to the elimination of virtually all T cells bearing V\(\beta\)-6, -8.1, and -3 (9-12).

There has been some discussion as to whether the survival of mice with the V\(\beta\) gene deletion or elimination of such a huge portion of their T cell repertoire is an artifact of the laboratory inbred mice. The laboratory strains presumably have to cope with a limited number of pathogens since they are maintained in relatively clean conditions. We set out to analyze the TCR usage of wild mice to determine
whether mice surviving under strong selective conditions also express and survive with depleted repertoires.

Our results showed a surprisingly uneven distribution of the TCR repertoire in the wild mice, with many of the mice homozygous for an extensive gene deletion and many examples of lowered expression of several Vβs, probably due to tolerance to self superantigens. Interestingly, Vβ8.2 expression was suppressed in Mls-1a mice. Since laboratory mouse strains have few Vβ8.2+ T cells with Mls-la reactivity (9), this finding facilitated the elucidation of the amino acids that contribute to Mls binding.

Materials and Methods

Mice. Wild mice were trapped at three independent sites around Gainesville, FL. All other mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

Cell Lines. T cell hybridomas were prepared by fusion of an αβ- derivative of the AKR thymoma BW5147 (13) to Con A-stimulated spleen cells expanded for 2 d in IL-2 (14). T cell hybridomas DO-II.10/S4.4 (15) and 3DT52.5 (16) were used as controls.

Stimulation Assays. Hybridomas were screened for reactivity to Mls-1a by stimulating 10⁵ of these cells with 10⁶ spleen cells from either CBA/J (Mls-1a) or B10.BR (Mls-1b). Lymphokine production was assayed after 24 h using the HT-2 cell line as an indicator (14).

Analyses of Vβ Expression. Lymph node T cells were prepared on nylon wool columns, while thymocytes were prepared and cultured for 3 h as previously described (6). These cells were stained with biotinylated anti-Vβ or anti-αβ antibodies followed by phycoerythrin streptavidin (PEAv; Tago Inc., Burlingame, CA) as outlined previously (6).

The panel of anti-Vβ antibodies used included anti-Vβ2, B20 (Malissen et al., unpublished observations), anti-Vβ3, KJ25 (11), anti-Vβ5, MR9-4 (Kanagawa et al., unpublished observations), anti-Vβ6, RR4-7 (17), anti-Vβ7, TR130 (Okada et al., unpublished observations), anti-Vβ8.1+8.2+8.3, P23.1 (18), anti-Vβ6.1+8.2, KJ16 (19), anti-Vβ6.2, F23.2 (18), anti-Vβ11, RR3 (20), and anti-Vβ17a, KJ23a (21). All Vβ levels are expressed as a percentage of cells bearing the TCR-α/β, as determined by staining with H57-597 (22).

Unseparated lymph node cells were stained with biotinylated anti-IE, 14.4.4 (23), and PEA. All stained cells were analyzed by using an Epics C flow cytometer as previously described (24).

Analyses of Vβ8 Gene Usage. Vβ8 gene usage by hybridomas was determined using Vβ8.1 leader (CTCTTCTTTTGTTTTGATT) and Vβ8.2 exon (CAGACTAATAACCACAA-CAAG)-specific oligonucleotides. Total RNA was prepared from 10⁷ hybrid cells. 1 μg RNA was used for the synthesis of cDNA using an antisense oligonucleotide specific for Cβ (GGCTACCCCTGTTGCTTTCGG) and reverse transcriptase (Amersham Corp., Arlington Heights, IL). The reaction was then heated for 5 min at 95°C before amplification of the cDNA using the discriminatory oligonucleotides specific for Vβ8.1 or Vβ8.2 shown above, the Cβ oligo, and 0.5 U of Taq polymerase (Perkin-Elmer Corp., Norwalk, CT). The following amplification conditions were provided by a thermocycler (Cetus Corp., Emeryville, CA); 95°C melting, 55°C annealing, and 72°C extension, each for 2 min.

Sequencing of Vβ8 genes. Total genomic DNA was prepared from liver. Vβ8 genes were equally amplified using the polymerase chain reaction and 1 μM external oligonucleotides (Vβ8.1, sense, CTCTTCTTTTGTTTTGATT and antisense, GAAATAGGGAAAACCAGTGCTT; Vβ8.2, sense, CTCTTCTTTGCCTCCTCCAGT and antisense, GAAATAGGGAAAACGTTGCAG) under the conditions described above. Subsequently, the gel-purified dsDNA was subjected to unequal amplification using 1 μM of external (see above) and 0.01 μM of internal oligonucleotides (Vβ8.1, sense, CACTGGTGTCTTTTCTTT and antisense, TGGCTTCTTCCACCTCTGCA; Vβ8.2, sense, CAGGTGTTCTCTTCCCTCCA and antisense, GGGTTTCTTCCACCTCTGCA) to generate single-stranded cDNA, again using the same conditions. The amplified positive or negative strand DNA was sequenced by the chain termination method (25) using the Sequenase kit from United States Biochemical Corp., Cleveland, OH.

Southern Analysis. Liver DNA was digested, subjected to electrophoresis, and transferred to nitrocellulose, as described by Mamiatis et al. (26). Filters were hybridized with Vβ probes
Results

41 wild mice (*Mus musculus domesticus*) were trapped at three independent sites around Gainesville, FL. Lymph node cells from these animals were stained with a mAb that reacts with all mouse IE molecules. All but one mouse expressed IE (data not shown). Purified lymph node T cells and thymocytes were stained with a collection of the available anti-Vβ antibodies and an antibody to all mouse α/β receptors (22). There are, at present, antibodies to only about half the murine Vβs and the percentages of peripheral and thymus α/β+ T cells bearing any of the detectable Vβs were determined.

Two mice had extraordinarily uneven Vβ expression. One contained 97% Vβ11+, and the other, 40% Vβ8.2+ T cells. This was probably a consequence of tumors, or recent exposure to an environmental superantigen (6 and Callahan et al., unpublished observations). These two animals were excluded from further analysis.

Fig. 1 shows a summary of the staining data for Vβ expression on peripheral T cells in the remaining 39 mice. To facilitate further discussion, the mice have been divided into four groups. The staining data for an individual mouse from each of these groups are shown in Table I.

Deletion of Vβ genes. T cells bearing a particular Vβ may be absent from the periphery either because of Vβ gene deletion or inactivation, or because of self super-

![Figure 1](https://example.com/f1.png)

**Figure 1.** Peripheral Vβ expression by wild mice. Purified lymph node T cells from 39 wild mice were stained with a panel of anti-Vβ antibodies. Vβ levels are expressed as a percentage of α/β+ cells as determined using H57-597 (22). CI, ER, and HS were three independent sites around Gainesville, FL, where the wild mice were trapped. (A) Mice homozygous for a deletion of Vβ genes. (B) Mls-1b mice with F23.2+, Vβ8.2+ cells. (C) Mls-1b mice with F23.2-, Vβ8.2+ cells. (D) Mls-1b mice with F23.2-, Vβ8.2+ cells. C57BL/6 mice were used as controls, and in six experiments, the mean ± SEM for the percentages of T cells expressing each Vβ in this strain were as follows: Vβ2, 6.0 ± 0.2; Vβ3, 3.8 ± 0.1; Vβ5, 6.7 ± 0.3; Vβ6, 7.9 ± 0.2; Vβ7, 3.5 ± 0.1; Vβ8.1, 7.4 ± 0.4; Vβ8.2, 10.7 ± 0.3; Vβ8.3, 6.6 ± 0.3; Vβ11, 5.7 ± 0.1.
UNEVEN DISTRIBUTION OF Vβ REPertoire IN WILD MICE

TABLE I

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Group*</th>
<th>B20 (2)</th>
<th>KJ25 (2)</th>
<th>MR9-4 (5.1 + 5.2)</th>
<th>RR4-7 (6)</th>
<th>TR130 (8)</th>
<th>F23.1 (AU 8a)</th>
<th>KJ16 (8.1 + 8.2)</th>
<th>F23.2 (8.2)</th>
<th>RR3 (11)</th>
<th>KJ23a (17a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI26</td>
<td>A</td>
<td>23.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.0</td>
<td>9.9</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>CI22</td>
<td>B</td>
<td>15.1</td>
<td>0.1</td>
<td>0.1</td>
<td>5.8</td>
<td>5.2</td>
<td>21.0</td>
<td>14.9</td>
<td>9.2</td>
<td>8.1</td>
<td>0.0</td>
</tr>
<tr>
<td>HS07</td>
<td>C</td>
<td>7.1</td>
<td>0.2</td>
<td>3.1</td>
<td>12.5</td>
<td>4.7</td>
<td>34.6</td>
<td>24.5</td>
<td>0.0</td>
<td>2.3</td>
<td>0.0</td>
</tr>
<tr>
<td>ER21</td>
<td>D</td>
<td>16.4</td>
<td>0.1</td>
<td>0.9</td>
<td>0.4</td>
<td>9.2</td>
<td>8.2</td>
<td>1.6</td>
<td>0.0</td>
<td>5.3</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Purified peripheral T cells were stained with the panel of available anti-Vβ antibodies (see Materials and Methods), and results are expressed as percent of all αβ+ T cells, as determined by staining with H57-597 (22).

* Groups are those assigned in Fig. 1.
† Vβ0.2 specificity was determined for laboratory mice (9).

10 of the mice, shown in group A (Fig. 1 A and Table I), had no Vβ5, -6, -8, antigen-mediated elimination in the thymus (6). These two mechanisms can be distinguished by examination of Vβ expression on thymocytes. Self superantigens eliminate almost all mature T cells and mature thymocytes expressing a particular Vβ, but only about half of immature thymocytes. Therefore, the presence or absence of particular Vβ on immature thymocytes can be used to distinguish between clonal elimination and gene deletion as mechanisms for inhibition of expression of a particular Vβ (Fig. 2).

![Figure 2](image-url)

**Figure 2.** Vβ6 expression by thymocytes from wild mice. Thymocytes were cultured for 3 h before staining with anti-TCR reagents. Percentages refer to the fraction of αβ+ T cells. KJ23a was used here as a negative control (dotted line).
or -11-bearing T cells in their peripheral lymph nodes and, moreover, had no thymocytes expressing these V\(\beta\)s (Fig. 2 C). Southern blot analysis of liver DNA from these mice confirmed that the genes for some of these V\(\beta\) elements were absent (Fig. 3). These mice had deleted all the members of the V\(\beta\)5 and V\(\beta\)8 gene families and V\(\beta\)6, -9, -11, -12, -13, and -15, which is at least half of the mouse V\(\beta\) genes. Comparison of these data with a V\(\beta\) gene map (31) indicated that the deletion began upstream of V\(\beta\)5 and extended over at least 100 kb to a point downstream of V\(\beta\)15.

The V\(\beta\) deletion in these Floridian mice was not the same as those previously reported for laboratory mice. Strains of the V\(\beta^a\) haplotype, SJL, SWR, C57L, C57BR (3), and AU SS/J (4), carry a deletion extending from upstream of V\(\beta\)5 to downstream of V\(\beta\)9. Unlike the Floridian mice, these laboratory mice contain and express V\(\beta\)6 and V\(\beta\)15. Recently, another V\(\beta\) gene deletion has been reported in the inbred strain, RIII S/J (5). This deletion includes V\(\beta\)17 and so extends one V\(\beta\) gene further downstream than the deletion carried by the wild mice of this study.

Chromosomes carrying the V\(\beta\) gene deletion were only detected in the CI and ER populations. Southern blots of DNA from mice in these populations were used to find out which of the mice that contained T cells expressing V\(\beta\)s included in the deletion were in fact heterozygous for the deleted chromosome. Heterozygotes were distinguished from mice homozygous for the nondeleted V\(\beta\) locus by comparing

![Figure 3](image)

**Figure 3.** V\(\beta\) gene deletion extends from downstream of V\(\beta\)1 to downstream of V\(\beta\)15. Liver DNA from BALB/c, SJL, and wild mice ER36 and CI09 (Fig. 1, group A) was digested to completion with Eco RI and Hind III. V\(\beta\)1, V\(\beta\)8, and V\(\beta\)15 probes were used on Southern blots of Eco RI digests, and a V\(\beta\)17 probe was used on a blot of the Hind III digest.
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the intensities of Vβ8.1 and Vβ8.2 bands with the intensity of the nonpolymorphic Vβ1 band (data not shown). The results of this analysis showed that the gene frequency of the Vβ deletion was 0.56 and 0.36 in the CI and ER populations, respectively. The fact that both populations contained individuals homozygous for the deleted chromosome (41% in CI and 9% in ER) indicated that this reduction in the Vβ repertoire was a competitive phenotype at both trapping sites.

None of the mice analyzed expressed Vβ17a, as determined by thymic staining with KJ23a (21). However, the wild mice had a gene hybridizing with a Vβ17 probe that showed the same Vβ17 restriction fragment-length polymorphism as BALB/c (Fig. 3). This pattern has recently been shown to be indicative of a pseudogene generated by a premature stop codon (32). Therefore, the wild mice probably contained the nonfunctional Vβ17b allele of this gene.

Further analysis of Vβ and Cβ polymorphisms (data not shown) demonstrated that there are at least three chromosomes segregating in these wild populations that have not been previously documented for laboratory inbred strains. These will be the subject of future investigations.

Self Superantigens Shape the T Cell Repertoire. It has recently been demonstrated that laboratory mice expressing the Mls-1a allele eliminate thymocytes bearing Vβ6, Vβ8.1, and Vβ9 (9, 10, 33), while those expressing Mls-2a and/or Mls-3a eliminate their Vβ3* T cells (11, 12). Fig. 1 (B–D) shows that elimination patterns reminiscent of those seen for Mls-1a-bearing inbred strains also occur in wild mouse populations. Mice containing high levels of Vβ6- and Vβ8.1-bearing T cells, animals which were presumably Mls-1b, are shown in Fig. 1, B and C. Presumed Mls-1a-expressing mice, which expressed low levels of peripheral T cells bearing Vβ-6, -8.1, and -8.2, are shown in Fig. 1 D. The deletion of Vβ8.2 cells will be discussed below.

Of the 39 mice, expression of Mls-1a could be examined in only 29, since deletion of the genes for Vβ6 and the Vβ8 precluded a test in 10 of the mice (Fig. 1 A). Nevertheless, Mls-1a appeared to be expressed with the reasonable frequency of 8 of 29 in the mice we could examine. There was some indication that expression of Mls-1a was population specific, because the gene was expressed only in animals from the ER site (Table I). Whether this is a significant finding, or an artifact of the relatively small numbers of animals we have tested, awaits further investigation.

Clonal elimination of T cells bearing Vβ3 was found in all four of the groups of mice shown in Fig. 1. It was striking that as many as 32 of the 39 mice we examined expressed Vβ3 with low frequency on peripheral T cells (<2%), presumably due to tolerance induced by Mls-2a or Mls-3a (11, 12) (Table II).

Other examples of clonal elimination of T cells reactive to self superantigens have been reported. Vβ5, -11, and -12 bearing T cells, like those bearing Vβ17a, are eliminated in mice expressing IE (8, 20, 34, 35). We found examples of these phenomena in the wild mice. Thus, of those mice in which it could be analyzed, 21 of 29 had low levels of Vβ5* T cells (<2%) and only two mice had Vβ5 levels >5% (Table II). However, despite the fact that all but one of the wild mice expressed IE, the majority of the mice in which it could be analyzed expressed high levels of Vβ11* T cells (Table II). Moreover, there was no correlation with IE expression for the few mice that did have low Vβ11 levels. Therefore, Vβ11 expression in the wild mice did not seem to be controlled by the same elements as in laboratory animals.
Table II

**Gene Deletion, Gene Mutation, and Self Superantigens Eliminate**

<table>
<thead>
<tr>
<th>Vβs from the Repertoire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Deletion</td>
</tr>
<tr>
<td>Gene Mutation</td>
</tr>
<tr>
<td>Mls-1*</td>
</tr>
<tr>
<td>Mls-2+/3*</td>
</tr>
<tr>
<td>IE + &quot;X&quot;</td>
</tr>
<tr>
<td>IE + &quot;Y&quot;</td>
</tr>
</tbody>
</table>

* Vβ-5, -6, -8, and -11 are included in the gene deletion, and so the effects of the self superantigens on these Vβs could only be assessed in the mice that had the full complement of genes.

Mls-reactive Vβ8.2+ T Cells. An altered Vβ8.2 gene product was detected in mice from the ER and HS populations, shown in Fig. 1, C and D. This Vβ8.2 element did not bind the F23.2 antibody, which is specific for Vβ8.2 elements of laboratory mouse strains (9), although it did bind the KJ16 antibody, specific for Vβ8.1 and -8.2 of laboratory mice (Table I). Southern blot analysis of Eco RI digests of liver DNA from mice with this altered staining pattern showed a Vβ8.1 band at the same position (4.4 kb) as in laboratory mice, but a smaller Vβ8.2 band (0.27 kb), as shown in Fig. 4.

As mentioned above, the presumed Mls-1a mice (Fig. 1 D) eliminated F23.2-, Vβ8.2+ T cells, in addition to those bearing Vβ6 and -8.1. This result was unexpected because in laboratory inbred strains the majority of Vβ8.1+ T cells are Mls-1a reactive and are eliminated in mice bearing this self superantigen, while few Vβ8.2+ T cells show Mls-1a reactivity (9).

To confirm the Mls reactivity of these F23.2-, Vβ8.2+ cells, hybridomas were generated from spleen cells of a mouse that expressed the variant Vβ8.2+ but lacked Mls-1a, ER33 (group C). All but one of the Vβ8.1+ or Vβ8.2+ KJ16-binding T cell hybrids from this fusion reacted to Mls-1a stimulation (Table III). Vβ usage by the hybrids was analyzed by using Vβ8.1- and -8.2-specific oligonucleotides and the polymerase chain reaction.

Table III shows that all the F23.2-, Vβ8.2+ hybrids generated from ER33 (group C) responded to Mls-1a. Only 50% of the hybrids were reactive from a fusion of spleen cells from CI02 (group B), which bore a F23.2+, Vβ8.2 product. Mls-1a was not expressed in the mice from site CI (Table II), and so the reactivity of this Vβ8.2 could not be assessed by analyzing the peripheral levels of Vβ8.2+ cells in these mice. We have previously shown that only 13% of Vβ8.2+ hybrids from B10.BR mice were Mls-1a reactive, while 79% of Vβ8.1+ hybrids from these mice showed this reactivity (9).
Some wild mice have an altered Vβ8.2 gene. A Southern blot of Eco RI digests of liver DNA from a panel of wild mice was probed with a Vβ8 probe. The staining patterns for the mice are shown in Fig. 1. C109, group A; HS18 and CI18, group B; HS26 and ER37, group C; and ER35, group D.

It should be noted that the Mls-1a stimulation of the Vβ8.1- and Vβ8.2-bearing hybrids (Table III) and of Vβ6-bearing hybrids (data not shown) generated from the wild mice strengthens our earlier assumption that the deletion of Vβ6+, Vβ8.1+, and Vβ8.2+ T cells in these wild mice was due to tolerance to the Mls-1a antigen.

**TABLE III**

<table>
<thead>
<tr>
<th>Source of T cell hybrids</th>
<th>mAb binding</th>
<th>No. of hybrids</th>
<th>No. of Mls-1a-reactive hybrids</th>
<th>Percent Mls-1a-reactive hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KJ16</td>
<td>F23.2</td>
<td>Vβ</td>
<td></td>
</tr>
<tr>
<td>CI02</td>
<td>+</td>
<td>-</td>
<td>8.1*</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>8.2*</td>
<td>8</td>
</tr>
<tr>
<td>ER33</td>
<td>+</td>
<td>-</td>
<td>8.1†</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td>8.2†</td>
<td>13</td>
</tr>
</tbody>
</table>

T cell hybridomas were generated from spleen cells from CI02 (group B) and ER33 (group C). Con A-stimulated spleen cells were expanded in IL-2 before fusion to BW/α/β−. KJ16+ hybrids were selected, and tested for Mls-1a reactivity by stimulation with CBA/J spleen cells and with B10.BR spleen cells as a haplotype-matched Mls-1a control.

* Vβ assignments were made by staining with F23.2 (anti-Vβ8.2).

† Vβ assignments were made by preparing total RNA, generating cDNA using a Cβ-specific oligonucleotide and reverse transcriptase, and by using Vβ8.1-, Vβ8.2-, and Cβ-specific oligonucleotides and the polymerase chain reaction to amplify the Vβ8 genes. Amplified DNA was run out on a 0.7% agarose gel and was visualized using ethidium bromide.
The PCR-amplified $\beta_8.1$ and -8.2 genes from some of the wild mice were sequenced and are shown in Fig. 5. The altered $\beta_8.2$ gene of cells that did not bind F23.2 and that were Mls-1a reactive contained five amino acid substitutions, which distinguished it from conventional $\beta_8.2$. One of these changes, asparagine to aspartic acid at position 22, was shared by the wild mouse F23.2+ $\beta_8.2^+$ cells (group B), 50% of which were Mls-1a reactive. Therefore, this amino acid may contribute to Mls reactivity by some of these cells. Two other amino acid substitutions (asparagine to serine at 8 and glycine to aspartic acid at 51) are changes that convert $\beta_8.2$ residues into those found in the same position in $\beta_8.1$, the Mls-1a-reactive member of the $\beta_8$ family in laboratory inbred mice. Therefore, these may also contribute to increased Mls reactivity. The remaining amino acid substitutions at positions 70 and 71 are unique to this wild $\beta_8.2$ gene. It is the two base changes at position 71 that generate the new Eco RI site in this altered $\beta_8.2$ gene (Fig. 4).

**Discussion**

It is not possible to estimate the size of the mouse $\alpha/\beta$ T cell repertoire accurately. The repertoire is probably on the order of $10^{10}$ different receptors, but is reduced...
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in individual animals by the phenomena of positive selection and tolerance (36–38). A first thought might suggest that evolution would select animals with the largest T cell repertoires. Such animals would presumably be able to recognize and deal with more environmental pathogens.

It was therefore surprising to discover that all mice did not act to maximize the total number of useful TCRs they could express. Some laboratory mouse strains lack the genes for about half the mouse VBs (3–5), an observation that was also found for some wild mice trapped in the Orkneys, Scotland (39). Another mouse strain lacks half the possible JBs (40). Other laboratory mice limit their total T cell repertoire in an unexpected way by self tolerance. Of course, tolerance to self is an all important prerequisite of a useful immune system, and immunologists have always assumed that the repertoire of lymphocytes would be somewhat limited because cells specific for self would be eliminated or suppressed. It was a surprise, however, to find out how dramatic the effects of tolerance could be, and that the products of single genes, called self superantigens, could lead to the elimination of substantial portions of the T cell repertoire.

It is possible that repertoire restriction of this type is permitted in laboratory mice, which lead a relatively pathogen-free life, but might not be frequently observed in wild animals under constant challenge from environmental pathogens. To test this idea we screened a collection of wild mice, trapped in three independent locations in Florida, both for genetic deletion of VBs, and for self superantigen-mediated clonal elimination of T cells bearing particular VBs.

The Floridian animals were frequently homozygous for a large deletion in the VB locus, encompassing 12 of the known VBs. The deletion-carrying chromosome found in these mice was not the same as that in SJL or RIII or other laboratory strains, because it included two VBs not deleted in SJL mice, and differed from the RIII chromosome 6 by the presence of a VB17 gene, albeit, as the nonfunctional VB17b allele. The existence of these three independent extensive gene deletions within the VB locus suggests that this deletion is not deleterious.

Additionally, the wild mice expressed VB-eliminating self superantigens with high frequency (Table II). Virtually no animal expressed high levels of VB5+ T cells, even though more than two-thirds of the mice contained functional VB5 genes. VB3-bearing T cells were also eliminated at high frequency by self superantigens. Less frequently, VB6+ and VB8.1+ cells were eliminated by, presumably, expression of Mls-1a. The wild mice had, in fact, found a way to eliminate an additional VB, VB8.2, the expression of which is not affected by self superantigens in laboratory strains. The selection of this additional VB with reactivity to the self superantigen, Mls-1a, strengthens the argument that it is advantageous to maintain variation in the T cell repertoire using polymorphic self superantigens.

On the other hand, some VBs were unexpectedly expressed in the wild mice, particularly VB11. Laboratory animals expressing IE eliminate VB11-bearing cells (20, 34, 35). This did not occur in the wild mice we tested, even though almost all of them expressed IE. Perhaps this result is indicative of an altered VB11 gene in the wild population, a possibility that will be examined in the future.

Overall, these data show that the mouse populations examined contained T cells able to express nearly all mouse VBs, but that each individual mouse was able to use only a subset of all these VBs as part of its TCRs. This suggests that expression
of all Vβs is not evolutionarily preferred for individual mice, an unusual evolutionary
gambit, which is reminiscent of MHC gene expression. For MHC genes also, indi-

dividual mice or men are limited in the total number of genes they can express,
two at each allele, although the population at large has considerable diversity.

What evolutionary mechanisms may be responsible for the maintenance of vari-

ability in Vβ expression? We suggest that two opposing selective pressures are re-

sponsible for this phenomenon. The deleterious effects of bacterial toxins, such as the

staphylococcal toxins, may select for individuals with reduced Vβ repertoires. The

staphylococcal toxins are powerful Vβ-specific T cell-stimulating superantigens in

mouse and man (6, 7, 41). Unpublished experiments have shown that laboratory

mice containing normal numbers of T cells, but lacking those with which a partic-

ular toxin can interact, are resistant to the pathogenic effects of that toxin. A similar

phenomenon may allow toxin resistance in wild mice, thus favoring individuals with

repertoires lacking particular Vβs.

The toxin-mediated selection for fewer Vβs may be counterbalanced by selection

for immune responsiveness against other endemic pathogens infecting natural mouse

populations. Parasitic antigenicity is capable of rapid evolution (42), and the notion

that parasites are selected for expression of antigenicity that occupies blind spots

in the immune responsiveness of their hosts has been postulated many times (reviewed

in references 43 and 44). Consequently, individuals with larger Vβ repertoires, and

therefore fewer blind spots, would be predicted to be favored during interactions

with pathogens other than those producing superantigens.

Control of Vβ expression at numerous independently segregating genetic loci,

Vβ, MHC, and the self superantigens, has the additional advantage of shuffling the

Vβ repertoire in individual mice, such that numerous Vβ phenotypes are generated

within a given mouse population. This shuffling in Vβ usage patterns randomizes

the patterns of immune response blind spots expressed among individuals. Thus,

pathogens with antigenicity that exploits one blind spot would not be at an advan-
tage when infecting individuals within the same deme with different blind spots,
a phenomenon that will blunt the effectiveness of this mode of pathogen evolution.

Several other points emerge from the data presented in this paper. First, there

was some evidence that Vβ expression was under different pressures at the different

sites where mice were trapped. As noted above, for example, Mls-1a expression, and

concomitant elimination of Vβ6, -8.1, and -8.2+ T cells, was only found in animals

from one site (Table II). Likewise, mice homozygous for the Vβ gene deletion were

found primarily at one site.

Second, the alterations in Vβ8.2 that convert these receptors to Mls-1a reactivity

may give some indication of the sites on Vβ with which self superantigens react.
The TCR model proposed by Chothia et al. (45) predicts that four of the amino

acid substitutions in the altered Vβ8.2 would occur in framework regions of the

receptor. However, aspartic acid at position 22 would be an exposed charged res-
due, close to the predicted antigen/MHC-binding site, and so may contribute to
the 50% Mls reactivity of the Vβ8.2 receptors of the wild mice in group B, which
have this single amino acid substitution. The aspartic acid for glycine substitution
at position 51 falls in a predicted complementarity determining region forming part
of the antigen/MHC-binding site of the TCR. Moreover, there is an aspartic acid
at this site in the Mls-1a-reactive, Vβ8.1 sequence (46), so this residue may well con-
tribute to the strong Mls-1α reactivity of the variant Vβ8.2. It should be noted, however, that this position is not an aspartic acid in another Mls-1α-reactive Vβ, Vβ6.

Summary

We have examined TCR Vβ expression in a collection of wild mice. Many of the mice were homozygous for a large deletion at the Vβ locus, and many animals also suppressed expression of several Vβs using self superantigens. Expression of Vβ8.2 was unexpectedly suppressed by a self superantigen in some wild mice, which was due to the presence in these animals of a variant Vβ8.2 gene. The amino acid changes in this gene product suggest contact sites between Vβ and the superantigen.

Although all Vβs are expressed within each wild mouse population, individual mice have a limited and variable Vβ repertoire. The independent origin of multiple Vβ deletions and the presence of polymorphic self superantigens suggest that this variation may be maintained by balancing selection.

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