SURPRISINGLY UNEVEN DISTRIBUTION OF THE T CELL RECEPTOR Vβ REPertoire IN WILD MICE

By ANN M. PULLEN,*1 WAYNE POTTS,§ EDWARD K. WAKELAND,§ JOHN KAPPLER,*11 AND PHILIPPA MARRACK*1111

From the *Howard Hughes Medical Institute at Denver, 1Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206; the §Department of Pathology, College of Medicine, University of Florida, Gainesville, Florida 32610; and the 1Departments of Biochemistry, Biophysics, and Genetics, and the 1Departments of Microbiology, Immunology, and Medicine, University of Colorado Health Sciences Center, Denver, Colorado 80206

Heterodimeric TCR-α/βs are made up of combinations of V, D, J, and C elements. The majority of laboratory inbred mouse strains are of the Vβ6 haplotype and have at least 20 Vβ genes from which to construct TCRs (1, 2); however, a number of strains have been reported to have deletions of large portions of the Vβ 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 (Vα, Jα, Vβ, Dβ, and Jβ) contribute to binding of a conventional antigen-MHC complex. A second group of antigens that stimulate T cells via their TCR Vβ 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 ~30% of their T cell repertoire because the expression of Mls-1a and Mls-2a or -3a in CBA/J animals leads to the elimination of virtually all T cells bearing Vβ-6, -8.1, and -3 (9-12).

There has been some discussion as to whether the survival of mice with the Vβ 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-1a 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-11.10/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-Vβ8.1+8.2, KJ16 (19), anti-Vβ8.2, F23.2 (18), anti-Vβ11, RR3 (20), and anti-Vβ17a, KJ25a (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 PEAv. All stained cells were analyzed using an Epics C flow cytometer as previously described (24).

**Analyses of Vβ Gene Usage.** Vβ gene usage by hybridomas was determined using Vβ8.1 leader (CTCTTCTTTTGTGGTTTTGATT)- and Vβ8.2 exon (CAGACTAATAACCACAA-GAGAACAAAGTGC) 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 (GGCTACCCTCGTGTGCTTGGC) 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 Vβ 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, CTCTTCTTTTGTGGTTTTGATT and antisense, GAAATAAGGAAAAC-TAGCTT; Vβ8.2, sense, CTCTTCCCCGTGCTCTCCAGT and antisense, GAAATAAGGAAAAAGTGC) 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, CACTGGTGCTGCTTTCTT and antisense, TGGCTTCCCCTACTCTGCACA; Vβ8.2, sense, CAGGGTGCTTCTCTTCACA and antisense, GGGTTTCCTCCCTTCAGCACA) 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 Maniatis et al. (26). Filters were hybridized with Vβ probes.
Vol(Palmer and Yague, unpublished observations), Va6(27), Va8, -10, and -15 (28), V017 (21), and Ca(29), and were labeled by random priming (30). The two final stringency washes were in 0.1% SSC, 0.1% SDS at 55°C for 30 min.

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.** 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

| Mouse | Group | B20 (2) | KJ25 (3) | MR9-4 (5.1 + 5.2) | RR4-7 (6) | TR130 (7) | F23.1 (AU 8a) | KJ16 (8.1 + 8.2) | F23.2 (8.2) | RR3 (11) | KJ23a (17a) |
|-------|-------|---------|----------|-------------------|---------|----------|-------------|----------------|-------------|----------|------------|
| C126  | A     | 23.0    | 0.7      | 0.0               | 9.9     | 0.0      | 0.0         | 0.0            | 0.0         | 0.0      | 0.0        |
| C122  | B     | 15.1    | 0.1      | 0.1               | 5.8     | 5.2      | 21.0        | 14.9           | 9.2         | 8.1      | 0.0        |
| HS07  | C     | 7.1     | 0.2      | 3.1               | 12.5    | 4.7      | 34.6        | 24.5           | 0.0         | 2.3      | 0.0        |
| ER21  | D     | 16.4    | 0.1      | 0.9               | 0.4     | 9.2      | 8.2         | 1.6            | 0.0         | 5.3      | 0.0        |

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.
† Vβ8.2 specificity was determined for laboratory mice (9).

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).

10 of the mice, shown in group A (Fig. 1A and Table I), had no Vβ5, -6, -8,
or -11-bearing T cells in their peripheral lymph nodes and, moreover, had no thymocytes expressing these Vβs (Fig. 2C). Southern blot analysis of liver DNA from these mice confirmed that the genes for some of these Vβ elements were absent (Fig. 3). These mice had deleted all the members of the Vβ5 and Vβ8 gene families and Vβ6, -9, -11, -12, -13, and -15, which is at least half of the mouse Vβ genes. Comparison of these data with a Vβ gene map (31) indicated that the deletion began upstream of Vβ5 and extended over at least 100 kb to a point downstream of Vβ15.

The Vβ deletion in these Floridian mice was not the same as those previously reported for laboratory mice. Strains of the Vβa haplotype, SJL, SWR, C57L, C57BR (3), and AU SS/J (4), carry a deletion extending from upstream of Vβ5 to downstream of Vβ9. Unlike the Floridian mice, these laboratory mice contain and express Vβ6 and Vβ15. Recently, another Vβ gene deletion has been reported in the inbred strain, RIII S/J (5). This deletion includes Vβ17 and so extends one Vβ gene further downstream than the deletion carried by the wild mice of this study.

Chromosomes carrying the Vβ 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 Vβs included in the deletion were in fact heterozygous for the deleted chromosome. Heterozygotes were distinguished from mice homozygous for the nondeleted Vβ locus by comparing

---

**Figure 3.** Vβ gene deletion extends from downstream of Vβ1 to downstream of Vβ15. 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. Vβ1, Vβ8, and Vβ15 probes were used on Southern blots of Eco RI digests, and a Vβ17 probe was used on a blot of the Hind III digest.
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-1a, 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 II). 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. Per-
haplos this reflects the presence in the wild mouse population of variant Vβ11-, variant IE-, or associated variant superantigens.

**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 C102 (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).
FIGURE 4. Some wild mice have an altered V$\beta$8.2 gene. A Southern blot of Eco RI digests of liver DNA from a panel of wild mice was probed with a V$\beta$8 probe. The staining patterns for the mice are shown in Fig. 1. CI09, group A; HS18 and CI18, group B; HS26 and ER37, group C; and ER35, group D.

It should be noted that the Mls-1+ stimulation of the V$\beta$8.1- and V$\beta$8.2-bearing hybrids (Table III) and of V$\beta$6-bearing hybrids (data not shown) generated from the wild mice strengthens our earlier assumption that the deletion of V$\beta$6+, V$\beta$8.1+, and V$\beta$8.2+ T cells in these wild mice was due to tolerance to the Mls-1+ antigen.

| Source of T cell hybrids | mAb binding | No. of hybrids | No. of Mls-1+ reactive hybrids | Percent Mls-1+ reactive hybrids |
|--------------------------|-------------|----------------|-----------------------------|--------------------------------|
| CI02                     | +           | 8.1+           | 8                           | 100                            |
|                          | +           | 8.2+           | 4                           | 50                             |
|                          | +           | 8.1            | 9                           | 90                             |
|                          | +           | 8.2           | 13                          | 100                            |

Table III

| V$\beta$8.1+ and V$\beta$8.2+ T Cell Hybrids from Wild Mice Are Mls-1+ Reactive |

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/a/β−. KJ16+ hybrids were selected, and tested for Mls-1+ reactivity by stimulation with CBA/J spleen cells and with B10.BR spleen cells as a haplotype-matched Mls-1+ control.

* V$\beta$ assignments were made by staining with F23.2 (anti-V$\beta$8.2).

1 V$\beta$ assignments were made by preparing total RNA, generating cDNA using a C$\beta$-specific oligonucleotide and reverse transcriptase, and by using V$\beta$8.1-, V$\beta$8.2-, and C$\beta$-specific oligonucleotides and the polymerase chain reaction to amplify the V$\beta$8 genes. Amplified DNA was run out on a 0.7% agarose gel and was visualized using ethidium bromide.
The PCR-amplified Vβ8.1 and -8.2 genes from some of the wild mice were sequenced and are shown in Fig. 5. The altered Vβ8.2 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 Vβ8.2. One of these changes, asparagine to aspartic acid at position 22, was shared by the wild mouse F23.2+, Vβ8.2+ 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 Vβ8.2 residues into those found in the same position in Vβ8.1, the Mls-1a-reactive member of the Vβ8 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 Va8.2 gene. It is the two base changes at position 71 that generate the new Eco RI site in this altered Vβ8.2 gene (Fig. 4).

Discussion

It is not possible to estimate the size of the mouse α/β T cell repertoire accurately. The repertoire is probably on the order of $10^{10}$ different receptors, but is reduced...
UNEVEN DISTRIBUTION OF Vβ REPERTOIRE IN WILD MICE

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 Vβs (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 Jβs (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 Vβs, and for self superantigen-mediated clonal elimination of T cells bearing particular Vβs.

The Floridian animals were frequently homozygous for a large deletion in the Vβ locus, encompassing 12 of the known Vβs. 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 Vβs not deleted in SJL mice, and differed from the RIII chromosome 6 by the presence of a Vβ17 gene, albeit, as the nonfunctional Vβ17b allele. The existence of these three independent extensive gene deletions within the Vβ locus suggests that this deletion is not deleterious.

Additionally, the wild mice expressed Vβ-eliminating self superantigens with high frequency (Table II). Virtually no animal expressed high levels of Vβ5+ T cells, even though more than two-thirds of the mice contained functional Vβ5 genes. Vβ3-bearing T cells were also eliminated at high frequency by self superantigens. Less frequently, Vβ6+ and Vβ8.1+ cells were eliminated by, presumably, expression of Mls-1a. The wild mice had, in fact, found a way to eliminate an additional Vβ, Vβ8.2, the expression of which is not affected by self superantigens in laboratory strains. The selection of this additional Vβ 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 Vβs were unexpectedly expressed in the wild mice, particularly Vβ11. Laboratory animals expressing IE eliminate Vβ11-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 Vβ11 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 Vβs, but that each individual mouse was able to use only a subset of all these Vβs as part of its TCRs. This suggests that expression
of all V\(\beta\)s is not evolutionarily preferred for individual mice, an unusual evolutionary gambit, which is reminiscent of MHC gene expression. For MHC genes also, individual 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 variability in V\(\beta\) expression? We suggest that two opposing selective pressures are responsible for this phenomenon. The deleterious effects of bacterial toxins, such as the staphylococcal toxins, may select for individuals with reduced V\(\beta\) repertoires. The staphylococcal toxins are powerful V\(\beta\)-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 particular 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 V\(\beta\)s.

The toxin-mediated selection for fewer V\(\beta\)s 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 V\(\beta\) repertoires, and therefore fewer blind spots, would be predicted to be favored during interactions with pathogens other than those producing superantigens.

Control of V\(\beta\) expression at numerous independently segregating genetic loci, V\(\beta\), MHC, and the self superantigens, has the additional advantage of shuffling the V\(\beta\) repertoire in individual mice, such that numerous V\(\beta\) phenotypes are generated within a given mouse population. This shuffling in V\(\beta\) 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 advantage 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 V\(\beta\) expression was under different pressures at the different sites where mice were trapped. As noted above, for example, Mls-1\(^a\) expression, and concomitant elimination of V\(\beta\)6, -8.1, and -8.2\(^+\) T cells, was only found in animals from one site (Table II). Likewise, mice homozygous for the V\(\beta\) gene deletion were found primarily at one site.

Second, the alterations in V\(\beta\)8.2 that convert these receptors to Mls-1\(^a\) reactivity may give some indication of the sites on V\(\beta\) 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 V\(\beta\)8.2 would occur in framework regions of the receptor. However, aspartic acid at position 22 would be an exposed charged residue, close to the predicted antigen/MHC-binding site, and so may contribute to the 50% Mls reactivity of the V\(\beta\)8.2 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-1\(^a\)-reactive, V\(\beta\)8.1 sequence (46), so this residue may well con-
tribute to the strong Mls-1* reactivity of the variant Vβ8.2. It should be noted, however, that this position is not an aspartic acid in another Mls-1*-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.

We thank Janice White, Rhonda Richards, and Terri Wade for their excellent technical assistance; Joel Boymel and Trinh Cao for preparation of oligonucleotides; and C. Jo Manning for assistance with the wild mouse population. We also thank Drs. Kanagawa, Malissen, Okada, Staerz, and Bevan for kindly making their antibodies available.

Received for publication 23 August 1989.

References

1. Barth, R. K., B. S. Kim, N. C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenson, C. Okada, D. Hansburg, K. L. Weissman, and L. Hood. 1985. The murine T cell receptor uses a limited repertoire of expressed Vβ gene segments. Nature (Lond.). 316:517.
2. Behlke, M. A., D. G. Spinella, H. S. Chou, W. Sha, D. L. Hardt, and D. Y. Loh. 1985. T cell receptor β chain expression: dependence on relatively few variable region genes. Science (Wash. DC). 229:566.
3. Behlke, M., H. Chou, K. Huppi, and D. Loh. 1986. Murine T cell receptor mutants with deletions of β-chain variable region genes. Proc. Natl. Acad. Sci. USA. 83:767.
4. Haqqi, T. M., S. Banerjee, W. L. Jones, G. Anderson, M. A. Behlke, D. Y. Loh, H. S. Luft, and C. S. David. 1989. Identification of T cell receptor Vβ deletion mouse strain AU/sj (H-2b) which is resistant to collagen-induced arthritis. Immunogenetics. 29:180.
5. Haqqi, T. M., S. Banerjee, G. Anderson, and C. S. David. 1989. RIII S/J (H-2d). An inbred mouse strain with a massive deletion of T cell receptor Vβ genes. J. Exp. Med. 169:1903.
6. White, J., A. Herman, A. Pullen, R. Kubo, J. W. Kappler, and P. Marrack. 1989. The Vβ-specific superantigen Staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. Cell. 56:27.
7. Janeway, C. A., Jr., J. Yagi, P. Conrad, M. Katz, S. Vroegop, and S. Buxser. 1989. T cell response to Mls and to bacterial proteins that mimic its behavior. Immunol. Rev. 107:61.
8. Kappler, J. W., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. Cell. 49:273.
9. Kappler, J. W., U. Staerz, J. White, and P. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. Nature (Lond.). 332:35.
10. MacDonald, H. R., R. Schneider, R. K. Lees, R. C. Howe, H. Acha-Orbea, H. Festen-
stein, R. M. Zinkernagel, and H. Hengartner. 1988. T cell receptor Vβ use predicts reactivity and tolerance to Mls*-encoded antigens. Nature (Lond.). 332:45.

11. Pullen, A. M., P. Marrack, and J. W. Kappler. 1988. The T cell repertoire is heavily influenced by tolerance to polymorphic self antigens. Nature (Lond.). 335:796.

12. Pullen, A. M., P. Marrack, and J. W. Kappler. 1989. Evidence that Mls-2 antigens which delete Vβ3+ T cells are controlled by multiple genes. J. Immunol. 142:3033.

13. Born, W., J. White, R. O'Brien, and R. Kubo. 1988. Development of T cell receptor expression: Studies using T cell hybridomas. Immunol. Res. 7:279.

14. Kappler, J., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, H-2-restricted, interleukin 2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J. Exp. Med. 153:1198.

15. White, J., K. Haskins, P. Marrack, and J. W. Kappler. 1983. Use of I region restricted, antigen specific T cell hybridomas to produce idiotypically specific anti-receptor antibodies. J. Immunol. 130:1033.

16. Endres, R., E. Kushnir, J. Kappler, P. Marrack, and S. Kinsky. 1983. A requirement for non-specific T cell factors in antibody responses to "T cell independent" antigens. J. Immunol. 130:781.

17. Kanagawa, O., E. Palmer, and J. Bill. 1989. T cell receptor Vβ6 domain imparts reactivity to an Mls antigen. Cell. Immunol. 119:412.

18. Staerz, U., H. Rammansee, J. Banedetto, and M. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. J. Immunol. 134:3994.

19. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. W. Kappler, and P. Marrack. 1984. The major histocompatibility complex-restricted antigen receptor on T cells. VI. An antibody to a receptor allotype. J. Exp. Med. 160:452.

20. Bill, J., O. Kanagawa, D. Woodland, and E. Palmer. 1989. The MHC molecule I-E is necessary but not sufficient for the clonal deletion of Vβ11-bearing cells. J. Exp. Med. 169:1405.

21. Kappler, J., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, N. 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.

22. Kubo, R. T., W. Born, J. W. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine αβ T cell receptors. J. Immunol. 142:2736.

23. Ozato, K., N. Mayer, and D. H. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J. Immunol. 124:533.

24. Roehm, N., L. Herron, J. Cambier, D. DiGuisto, K. Haskins, J. W. Kappler, and P. Marrack. 1984. The major histocompatibility complex restricted antigen receptor on T cells. VII. Distribution on thymus and peripheral T cells. Cell. 38:577.

25. Sanger, F., S. Nicklen, and A. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463.

26. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 382-389.

27. Garman, R., J. Ko, C. Vulpe, and D. Raulet. 1986. T cell receptor variable region gene usage in T cell populations. Proc. Natl. Acad. Sci. USA. 39:87.

28. Wilson, R. K., E. Lai, R. Concannon, R. K. Barth, and L. E. Hood. 1988. Structure, organization and polymorphisms of murine and human T cell receptor alpha and beta chain genes families. Immunol. Rev. 101:149.

29. Born, W., J. Yague, E. Palmer, J. Kappler, and P. Marrack. 1985. Rearrangement of T cell receptor β chain genes during T cell development. Proc. Natl. Acad. Sci. USA. 92:295.

30. Feinberg, A. P., and P. Vogelstein. 1985. Anal. Biochem. 122:13.
UNEVEN DISTRIBUTION OF Vβ REPERTOIRE IN WILD MICE

31. Lai, E., R. Barth, and L. Hood. 1987. Genomic organization of the mouse T-cell receptor β-chain gene family. *Proc. Natl. Acad. Sci. USA.* 84:3846.

32. 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.

33. Happ, M. P., D. Woodland, and E. Palmer. 1989. A third T cell receptor Vβ gene encodes reactivity to Mls-1a gene products. *Proc. Natl. Acad. Sci. USA.* 86:6293.

34. Tomonari, K., and E. Lovering. 1988. T cell receptor-specific monoclonal antibodies against a Vβ11-positive mouse T cell clone. *Immunogenetics.* 28:445.

35. Bill, J., V. B. Appel, and E. Palmer. 1988. An analysis of T cell receptor variable region gene expression in major histocompatibility complex disparate mice. *Proc. Natl. Acad. Sci. USA.* 85:9184.

36. Bevan, M. 1977. In a radiation chimera host H-2 antigens determine the immune responsiveness of donor cytotoxic cells. *Nature (Lond.)* 269:417.

37. Zinkernagel, R., 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.

38. Lederberg, J. 1959. Genes and antibodies. *Science (Wash. DC.)* 129:1649.

39. Huppi, K., B. D'Hoostelaere, B. Mock, E. Jouvin-Marche, M. Behlke, H. Chou, R. Berry, and D. Loh. 1988. T-cell receptor Vβ genes in natural populations of mice. *Immunogenetics.* 27:51.

40. Kotzin, B., V. Barr, and E. Palmer. 1985. A large deletion within the T-cell receptor beta-chain gene complex in New Zealand white mice. *Science (Wash. DC.)* 229:167.

41. Kappler, J. W., B. Kotzin, L. Herron, E. W. Gelfand, R. D. Bigler, A. Boylston, S. Carrel, D. Posnett, Y. Choi, and P. Marrack. 1989. Vβ-specific stimulation of human T cells by Staphylococcal toxins. *Science (Wash. DC.)* 244:811.

42. Buonagurio, D. A., S. Nakada, J. D. Parvin, M. Krystal, P. Palese, and W. M Fitch. 1986. Evolution of human influenza A viruses over 50 years: rapid, uniform rate of change in NS gene. *Science (Wash. DC.)* 232:980.

43. Schwartz, R. H. 1986. Immune response (Ir) genes of the murine major histocompatibility complex. *Adv. Immunol.* 38:31.

44. Damian, R. T. 1987. Molecular mimicry revisited. *Parasitol. Today.* 3:263.

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

46. Chou, H., S. Anderson, M. Louie, S. Godambe, M. Pozzi, M. Behlke, K. Huppi, and D. Loh. 1987. Tandem linkage and unusual RNA splicing of the T cell receptor β-chain variable-region genes. *Proc. Natl. Acad. Sci. USA.* 84:1992.