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

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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 $V\beta^b$ 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 $Mls\text{-}1^a$ and $Mls\text{-}2^a$ or $\text{-}3^a$ 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

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[1] *Abbreviation used in this paper:* Mls, mixed lymphocyte stimulating locus.

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-1^a mice. Since laboratory mouse strains have few Vβ8.2$^+$ T cells with Mls-1^a 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 3DT-52.5 (16) were used as controls.

Stimulation Assays. Hybridomas were screened for reactivity to Mls-1^a by stimulating 10^5 of these cells with 10^6 spleen cells from either CBA/J (Mls-1^a) or B10.BR (Mls-1^b). 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, F23.1 (18), anti-Vβ8.1+8.2, KJ16 (19), anti-Vβ8.2, 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 PEAv. 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 (CTCTTCTTTGTGGTTTTGATT)- and Vβ8.2 exon (CAGACTAATAACCACAACAAC)-specific oligonucleotides. Total RNA was prepared from 10^7 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 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, CTCTTCTTTGTGGTTTTGATT and antisense, GAAATAGGGAAAACTAGCTCT; Vβ8.2, sense, CTCTTCTTCGTGCTCTCCAGT and antisense, GAAATAAGGAGAACAAAGTGC) 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, CACTGGTGTGCTTTCTT and antisense, TGGCTTCCTTCACTCTGCACA; Vβ8.2, sense, CAGGTGTGCTTCTCTCTCCA and antisense, GGGTTTCCTCCCCTCTGCACA) 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

Vβ1 (Palmer and Yague, unpublished observations), Vβ6 (27), Vβ8, -10, and -15 (28), Vβ17 (21), and Cβ (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% $V\beta11^+$, and the other, 40% $V\beta8.2^+$ 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-

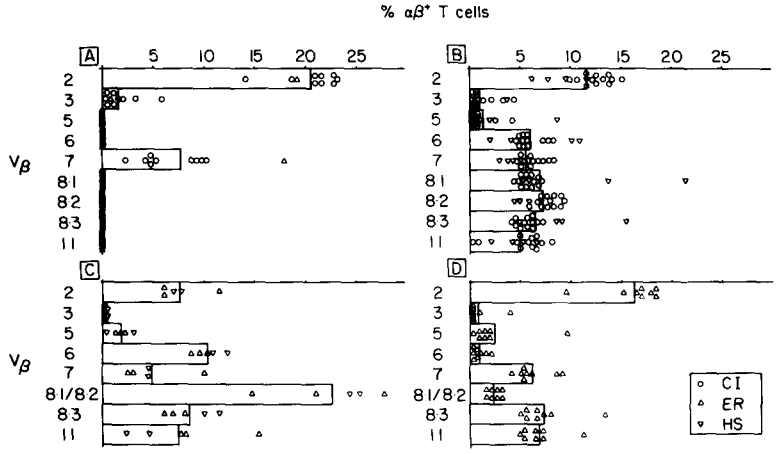

FIGURE 1. Peripheral Vβ expression by wild mice. Purified lymph node T cells from 39 wild mice were stained with a panel of anti-Vβ antibodies. Vβ levels are expressed as a percentage of α/β^+ cells as determined using H57-597 (22). CI, ER, and HS were three independent sites around Gainesville, FL, where the wild mice were trapped. (*A*) Mice homozygous for a deletion of Vβ genes. (*B*) Mls-1^b mice with $F23.2^+$, $V\beta8.2^+$ cells. (*C*) Mls-1^b mice with $F23.2^-$, $V\beta8.2^+$ cells. (*D*) Mls-1^a mice with $F23.2^-$, $V\beta8.2^+$ 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.

TABLE I

Individual Mice Express Different Vβ Repertoires

		Percent of peripheral T cells staining anti-Vβ mAbs (Vβ specificity)									
Mouse	Group*	B20 (2)	KJ25 (3)	MR9-4 (5.1 + 5.2)	RR4-7 (6)	TR130 (7)	F23.1 (AU 8s)	KJ16 (8.1 + 8.2)	F23.2 (8.2)‡	RR3 (11)	KJ23a (17a)
CI26	A	23.0	0.7	0.0	0.0	9.9	0.0	0.0	0.0	0.0	0.0
CI22	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 α/β^+ 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. 1 *A* and Table I), had no Vβ5, -6, -8,

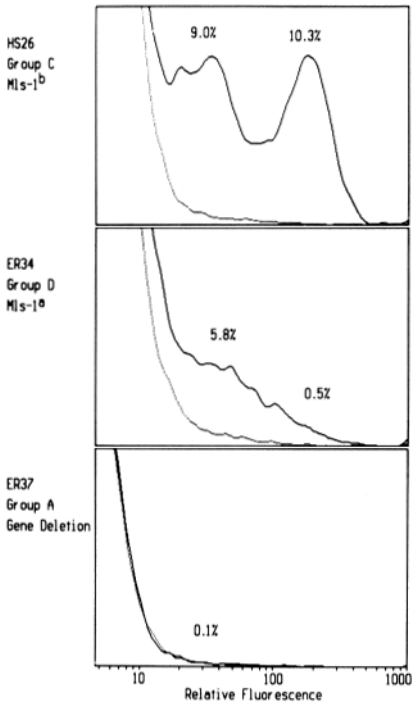

FIGURE 2. Vβ6 expression by thymocytes from wild mice. Thymocytes were cultured for 3 h before staining with anti-TCR reagents. Percentages refer to the fraction of α/β^+ T cells. KJ23a was used here as a negative control (*dotted line*).

or -11-bearing T cells in their peripheral lymph nodes and, moreover, had no thymocytes expressing these Vβs (Fig. 2 *C*). 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 $V\beta^a$ 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

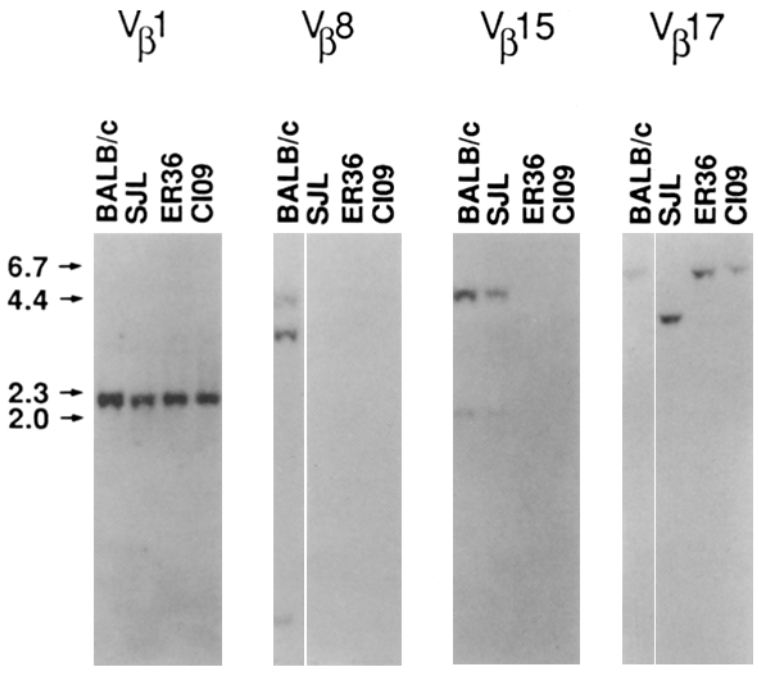

FIGURE 3. Vβ gene deletion extends from downstream of Vβ1 to downstream of Vβ15. Liver DNA from BALB/c, SJL, and wild mice ER36 and CI09 (Fig. 1, group A) was digested to completion with Eco RI and Hind III. Vβ1, Vβ8, and Vβ15 probes were used on Southern blots of Eco RI digests, and a Vβ17 probe was 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-1^a allele eliminate thymocytes bearing Vβ6, Vβ8.1, and Vβ9 (9, 10, 33), while those expressing Mls-2^a and/or Mls-3^a eliminate their Vβ3$^+$ T cells (11, 12). Fig. 1 (*B–D*) shows that elimination patterns reminiscent of those seen for Mls-1^a-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-1^b, are shown in Fig. 1, *B* and *C*. Presumed Mls-1^a-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-1^a 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-1^a 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-1^a 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-2^a or Mls-3^a (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-

TABLE II

Gene Deletion, Gene Mutation, and Self Superantigens Eliminate Vβs from the Repertoire

Vβ	Eliminating element	Mice with low peripheral Vβ levels			
		Site			
		CI	ER	HS	Total*
5, 6, 8, 9, 11, 12, 13, 15	Gene deletion	9/21	1/12	0/6	10/39
17	Gene mutation	21/21	12/12	6/6	39/39
6 and 8.1	Mls-1^a	0/12	8/11	0/6	8/29
3	Mls-2^a/3^a	16/21	11/12	5/6	32/39
5	IE + "X"	10/12	8/11	3/6	21/29
11	IE + "Y"	2/12	0/11	0/6	2/29

* 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.

haps 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-1^a 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-1^a reactive and are eliminated in mice bearing this self superantigen, while few Vβ8.2$^+$ T cells show Mls-1^a 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-1^a, ER33 (group C). All but one of the Vβ8.1$^+$ or Vβ8.2$^+$ KJ16-binding T cell hybrids from this fusion reacted to Mls-1^a 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-1^a. 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-1^a 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-1^a reactive, while 79% of Vβ8.1$^+$ hybrids from these mice showed this reactivity (9).

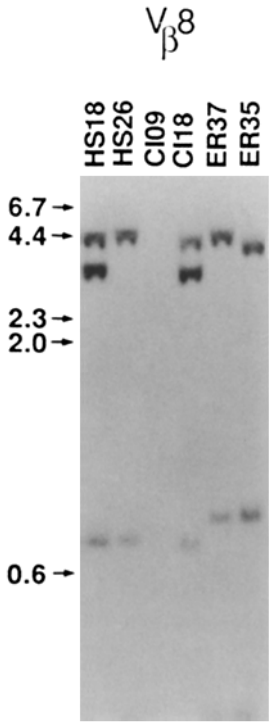

FIGURE 4. Some wild mice have an altered Vβ8.2 gene. A Southern blot of Eco RI digests of liver DNA from a panel of wild mice was probed with a Vβ8 probe. The staining patterns for the mice are shown in Fig. 1. CI09, group A; HS18 and CI18, group B; HS26 and ER37, group C; and ER35, group D.

It should be noted that the Mls-1^a 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-1^a antigen.

TABLE III

Vβ8.1$^+$ and Vβ8.2$^+$ T Cell Hybrids from Wild Mice Are Mls-1^a Reactive

Source of T cell hybrids	mAb binding KJ16	mAb binding F23.2	Vβ	No. of hybrids	No. of Mls-1^a-reactive hybrids	Percent Mls-1^a-reactive hybrids
CI02	+	–	8.1*	8	8	100
	+	+	8.2*	8	4	50
ER33	+	–	8.1‡	10	9	90
	+	–	8.2‡	13	13	100

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-1^a reactivity by stimulation with CBA/J spleen cells and with B10.BR spleen cells as a haplotype-matched Mls-1^b 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 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-1^a 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-1^a 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-1^a-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 Vβ8.2 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

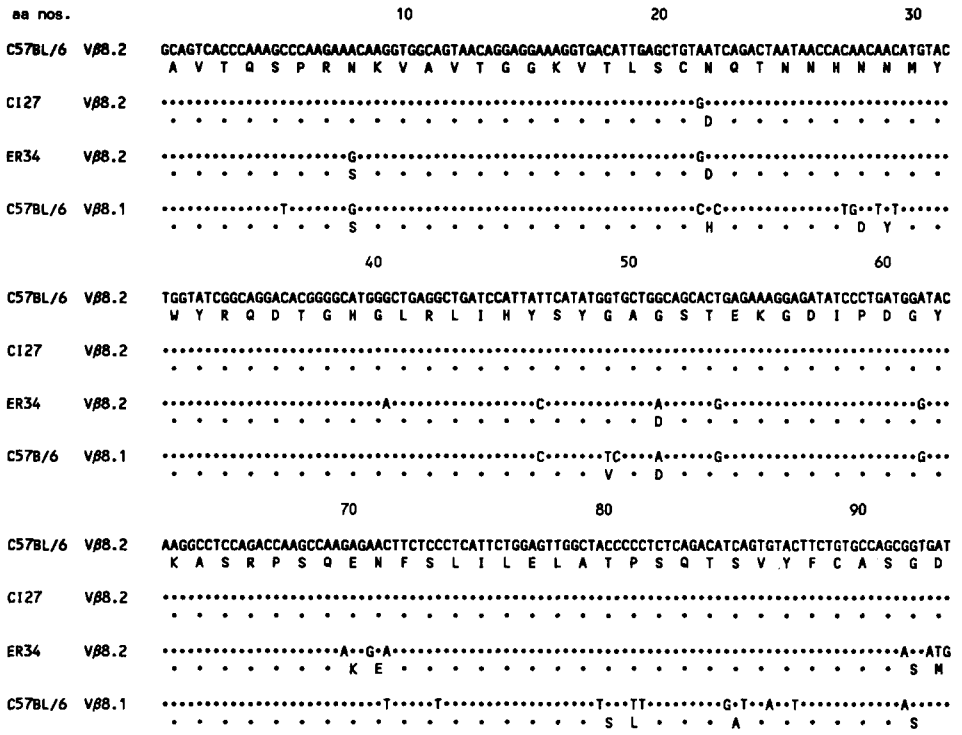

FIGURE 5. Sequences of Vβ8.2 genes from wild mice. The sequences shown for the Vβ8.1 and Vβ8.2 genes of C57BL/6 have been previously published (44). Oligonucleotides used for the amplification and sequencing of the wild mouse genes are shown in Materials and Methods. Sequence CI27 was identical to that obtained for CI19 (group B). Sequence ER34 was identical to that for ER14 and ER35 (group D), and differed at only one base (phenylalanine at position 72), from HS26 (group C).

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 $V\beta5^+$ 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, $V\beta6^+$ and $V\beta8.1^+$ cells were eliminated by, presumably, expression of $Mls\text{-}1^a$. 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, $Mls\text{-}1^a$, 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 Vβs 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 Vβ 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 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 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 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 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 Vβ 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 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-1^a 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 residue, 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-1^a-reactive, Vβ8.1 sequence (46), so this residue may well con-

tribute to the strong Mls-1^a reactivity of the variant Vβ8.2. It should be noted, however, that this position is not an aspartic acid in another Mls-1^a-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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