

ANALYSIS OF V β 17a EXPRESSION IN NEW MOUSE STRAINS BEARING THE V β ^a HAPLOTYPE

BY JOHN W. KAPPLER,*[†] ELLA KUSHNIR, AND PHILIPPA MARRACK*[†][§]

*From the Howard Hughes Medical Institute, Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206; and the Departments of *Medicine, of [†]Microbiology and Immunology, and of [§]Biochemistry, Biophysics, and Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262*

In the mouse the V β elements of the TCR- α/β are encoded in a complex of tightly linked genes on chromosome six (1-3). Two major V β haplotypes have been found. Most common strains carry the V β ^b haplotype, which encodes ~20 V β elements (4-8). A few strains carry the V β ^a haplotype, in which a large deletion has eliminated 10 V β gene elements (9). In addition, the V β ^a haplotype carries a functional gene for V β 17 (V β 17a) (10). A V β 17 gene is present in the V β ^b haplotype (V β 17b), but is inactive due to a mutation in the coding region that generates a termination codon (11).

We recently reported (10) that TCR- α/β that utilize V β 17a react with very high frequency to the murine MHC H-2 class II product, IE. This reactivity is demonstrated most dramatically in IE⁺ mice, where during the establishment of self tolerance, V β 17a⁺ T cells are virtually eliminated (12). This phenomenon has provided a useful tool to study the process of self-tolerance; however, experiments have been limited because only a few strains carry the V β ^a haplotype and these strains are of diverse genetic backgrounds, making it difficult to distinguish H-2 and non-H-2 effects on V β 17a expression.

We report here the characteristics of V β 17a expression in a set of new mouse strains constructed to have the V β ^a haplotype associated with various H-2 haplotypes in related C57 mice. The results not only confirm the association of the deletion of V β 17a⁺ T cells in IE⁺ mice, but also establish a second H-2-encoded ligand for V β 17a⁺ T cells mapping to the K end of the H-2^k haplotype, and suggest that CD4⁺ V β 17a⁺ T cells are differentially selected in mice of different H-2 types.

Materials and Methods

Mice. Mice were either bred in our own facility or purchased from the Jackson Laboratory, Bar Harbor, ME.

mAbs and Flow Cytometric Analyses. mAbs used to characterize purified peripheral T cells are listed in Table I. For use in flow cytometric analyses, in some cases, purified antibodies were labeled with FITC or biotin-N-hydroxysuccinimide by standard procedures. Biotinylated

This work was supported by grants AI-18785, AI-22259, and AI-17134 from the Department of Health and Human Services, National Institutes of Health. Address correspondence to John W. Kappler, Howard Hughes Medical Institute, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson St., Denver, CO 80206.

(Bio)¹ antibodies were detected with phycoerythrin-streptavidin (PEAv; Tago Inc., Burlingame, CA) as a secondary reagent. Unconjugated murine antibodies were detected with an FITC-labeled goat anti-mouse Ig secondary reagent (Calbiochem-Behring Corp., San Diego, CA). All samples were analyzed with an Epics C flow cytometer.

V β and H-2 Typing of Mice. Approximately 10 drops of peripheral blood were obtained from the tail artery of mice to be typed. RBC were removed with Gey's solution (20) and T cells enriched on nylon fiber columns (21, 22). Aliquots of cells were stained with anti-H-2 antibodies. For estimating T cell V β expression, cells were stained with FITC-anti-CD4 and FITC-anti-CD8 (separately or in combination) and simultaneously with Bio-anti-V β plus PEAv to estimate V β usage. The sum of CD4⁺ plus CD8⁺ T cells in these preparations varied from 75% to 90%. In some experiments mice were killed and T cells from lymph node cell suspensions were similarly prepared and stained. In this case the sum of CD4⁺ and CD8⁺ T cells varied from 95% to 100%.

TABLE I
mAbs Used in These Studies

mAb	Relevant specificity	Reference
MK-Q8	D ^{b,q}	10*
11-4.1	K ^k	13
34-2-12	D ^d	14
28-13-3	K ^b	15
GK-1.5	CD4	16
53.6	CD8	17
KJ23a	V β 17a	10
KJ16	V β (8.1 + 8.2)	18, 19

* Produced from the same fusion that yielded KJ23a. Typed on H-2 congenic mice.

Results

Production of New V β ^a Strains. We produced F₁ mice by crossing either C57BR(H-2^k) or C57L(H-2^b) mice to any of a number of H-2 congenic mice on the C57BL/10 (B10) background. The F₁ mice were crossed to produce F₂ progeny. T cells from these mice were typed for H-2 haplotype. Mice homozygous at H-2 were distinguished from heterozygous mice both with antibodies specific for the parental H-2 haplotypes and by the fact that individual T cells from heterozygous mice expressed approximately one half the amount of each parental H-2 haplotype. The T cells were also typed for V β haplotype, using KJ23a to detect V β 17a of the V β ^a haplotype and KJ16 to detect V β 8.1/8.2 carried only by the V β ^b haplotype. V β ^b homozygous mice were identified by the complete lack of V β 17a⁺ T cells (10–12) and a high level of V β 8⁺ T cells, similar to that seen in the original V β ^b homozygous parent of the cross. V β ^a/V β ^b heterozygous mice were identified by the presence of both V β 8⁺ and V β 17a⁺ T cells. In the case of V β 8, the level of expression was ~50% of that seen in the V β ^b homozygous parent of the cross, reflecting allelic exclusion in the V β complex in individual T cells (23). Levels of V β 17a in the heterozygous mice were similarly reduced by allelic exclusion and, in addition, in a number of cases by the presence of a tolerizing MHC product, such as IE. However, residual

¹ Abbreviations used in this paper: Bio, biotinylated; PEAv, phycoerythrin-streptavidin.

Vβ17a⁺ T cells were detectable in every case. The important Vβ^a homozygous mice were identified in every case by the complete absence of Vβ8⁺ T cells and the presence of at least some Vβ17a⁺ T cells, again even in the presence of a tolerizing MHC molecule. In fact, with no exception, staining with these anti-Vβ antibodies placed the several hundred mice examined in these studies unequivocally into one of these three categories.

Some of these points are illustrated in Fig. 1 for an experiment in which 29 (B10.A(5R) × C57L)F₂ mice were typed. Using antibodies to D^d and D^b to distinguish the two H-2 types, homozygous mice of both types were detected. H-2 heterozygous mice were also identified, reacting with both antibodies, but with cells expressing ~50% of the level of each compared with homozygous mice. For each of these three groups, mice homozygous for Vβ^b contained ~16-19% Vβ8.1/8.2⁺ T cells, while this percentage dropped about half to 9-10% in Vβ^b/Vβ^a heterozygous mice and to 0% in homozygous Vβ^a mice. In H-2^b mice a reciprocal pattern of expression was seen for Vβ17a with ~5% in Vβ^a homozygous mice, 2.5% in a Vβ^b/Vβ^a heterozygous mouse, and 0% in Vβ^b homozygous mice. Due to the presence of the IE^b molecule, ~80% of Vβ17a⁺ T cells were deleted in H-2 heterozygous mice and in those homozygous for the H-2 type of B10.A(5R). In this experiment one mouse was identified that was both homozygous for Vβ^a and for the H-2 type of B10.A(5R), and two Vβ^a/H-2^b homozygous mice were found. Similar results were obtained with mice from each of the other crosses.

Mice homozygous for Vβ^a and for H-2 were used as founder stock to establish a new Vβ^a strain. These mice occurred approximately with the predicted 1 in 16 frequency so that often a founder pair was identified after typing 30-50 F₂ mice. In some cases a male and a female double-homozygous pair was not isolated, so that one double-homozygous mouse was bred with a mouse homozygous at only one of the loci. The double-homozygous mice identified among the F₃ progeny with a frequency of ~50% were used to establish the strain. Once established, the strains were maintained by breeders taken whenever possible from a single litter. New breeders also were selected for black coat color in order to select for the eventual loss of the leaden and brown coat color genes of C57L and C57BR.

The resulting strains, while neither completely congenic nor inbred, differed at H-2 and only some of the genes that distinguish the closely related C57BL, C57BR, and C57L strains. To control partially for these differences, H-2^k/Vβ^a and H-2^b/Vβ^a

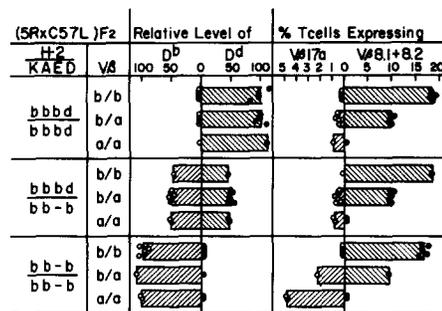


FIGURE 1. H-2 and Vβ typing of (B10.A(5R) × C57L)F₂ mice. T cells were prepared from the peripheral blood of 29 (B10.A(5R) × C57L)F₂ mice and typed for H-2 and Vβ as described in Materials and Methods and Results, staining with MK-Q8 (anti-D^b), 34-2-12 (anti-D^d), KJ23a (anti-Vβ17a), and KJ16 (anti-Vβ8.1+8.2). H-2 data are shown as the relative mean fluorescence intensity normalized to an average of 100 for T cells from the homozygous mice. Vβ data are expressed as the percent of total CD4⁺ plus CD8⁺ cells that stained with each of the anti-Vβ antibodies. Each point represents a determination made in an individual mouse.

TABLE II
Origins of New V β^a Mouse Strains

V β^a strain	H-2					Derived from:
	Type	K	A	E	D	
B10.Q β_{BR}	q	q	q	-	q	(B10.Q \times C57BR)F ₂ /F ₃
B10.S(7R) β_{L}	t2	s	s	-	d	(B10.S[7R] \times C57L)F ₂
B10 β_{L}	b	b	b	-	b	B10.HTT \times C57L)F ₂
B10. β_{J} (N6)	b	b	b	-	b	(C57BL/10 \times [C57BL/10 \times SJL]F ₁)F ₆
B10.A(4R) β_{L}	h4	k	k	-	d	(B10.A[4R] \times C57L)F ₂
B10.D2 β_{L}	d	d	d	d	d	(B10.D2 \times C57L)F ₂ /F ₃
B10.A(5R) β_{L}	i5	b	b	b	d	(B10.A[5R] \times C57L)F ₂ /F ₃
B10.HTT β_{L}	t3	s	s	s	d	(B10.HTT \times C57L)F ₂ /F ₃
B10.BR β_{BR}	k	k	k	k	k	(B10.BR \times C57BR)F ₂

mice produced by the same strategy were compared with C57BR and C57L, respectively, to see if mixing C57BL genes with those of C57BR or C57L mice altered expression of V β 17a. In the case of H-2^k, this also produced a strain that bred much better than C57BR.

An additional V β^a strain was produced by backcrossing the SJL-derived V β^a complex to C57BL/10 mice, again typing progeny with anti-H-2^b and anti-V β antibodies. At the F₆ generation males and females from the same litter were mated to produce an N6 H-2^b/V β^a homozygous strain.

The origins of the nine new V β^a strains are summarized in Table II.

Deletion of V β 17a⁺ T cells in IE⁺ V β^a Mice. The new V β^a strains were compared with existing V β^a strains for the expression of V β 17a in CD4⁺ and CD8⁺ T cells. Purified lymph node T cells were stained simultaneously with either FITC-labeled anti-CD4 or anti-CD8 and Bio-anti-V β 17a plus PEAv. Sample fluorescence histograms are shown in Fig. 2 for some of the strains. The summarized results of all

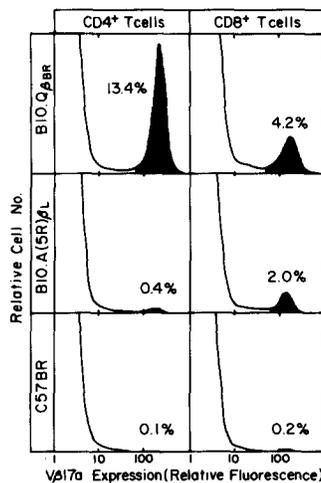


FIGURE 2. Deletion of V β 17a⁺ CD4⁺ and CD8⁺ T cells in IE⁺ mice. Nylon fiber-purified lymph node T cells from B10.Q β_{BR} , B10.A(5R) β_{L} , and C57BR mice were stained with either FITC-anti-CD4 or FITC-anti-CD8 and with Bio-anti-V β 17a plus PEAv. Histograms show the red fluorescence of the green fluorescent cells. Percentages refer to the percentage of total green fluorescence cells staining red in the shaded area.

TABLE III
Vβ17a Expression in Vβ^a Mice

Vβ ^a strain	H-2				T cells expressing Vβ17a*			
	Type	K	A	E	D	Percent of CD4 ⁺ T cells	Percent of CD8 ⁺ T cells	Percent of total T cells†
SWR	q	q	q	-	q	18.5 ± 0.6	4.6 ± 0.4	15.1 ± 0.6
B10.Qβ _{BR}	q	q	q	-	q	13.2 ± 0.5	3.7 ± 0.2	9.9 ± 0.4
SJL	s	s	s	-	s	10.9 ± 0.2	7.1 ± 0.3	9.8 ± 0.2
B10.S(7R)β _L	t2	s	s	-	d	5.0 ± 0.5	3.6 ± 0.1	4.3 ± 0.3
C57L	b	b	b	-	b	2.8 ± 0.2	6.3 ± 0.7	4.2 ± 0.4
B10β _L	b	b	b	-	b	3.1 ± 0.2	6.3 ± 0.3	4.1 ± 0.4
B10.βJ(N6)	b	b	b	-	b	3.4 ± 0.3	7.3 ± 0.2	4.9 ± 0.2
B10.A(4R)β _L	h4	k	k	-	b	1.7 ± 0.1	0.9 ± 0.0	1.3 ± 0.0
B10.D2β _L	d	d	d	d	d	1.3 ± 0.2	2.5 ± 0.3	1.8 ± 0.2
B10.A(5R)β _L	i5	b	b	b	d	0.5 ± 0.2	2.2 ± 0.5	1.1 ± 0.3
B10.HTTβ _L	t3	s	s	s	d	0.6 ± 0.1	1.1 ± 0.3	0.8 ± 0.2
B10.BRβ _{BR}	k	k	k	k	k	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1
C57BR	k	k	k	k	k	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0

* Average ± SEM of determinations made in three or more mice.

† Total T cells equals the sum of CD4⁺ plus CD8⁺ T cells.

the strains are listed in Table III and as the average percent of either CD4⁺, CD8⁺, or total T cells expressing Vβ17a.

As expected, Vβ17a⁺ T cells were depressed in all of the new Vβ^a strains bearing an IE molecule. That this low expression was due to tolerance to IE was confirmed by the dominant low expression in the Vβ^a homozygous/IE heterozygous F₂ and F₃ mice examined in the course of isolating these strains (Fig. 3). In each case the deletion of Vβ17a⁺ T cells was as striking in IE⁺ × IE⁻ heterozygous mice as in IE⁺ homozygous mice.

In our previous experiments it was difficult to compare the efficiencies of different IE molecules in this deletion because we had to use F₁ mice in which either the IE or the Vβ17a structural gene or both were heterozygous (12). However, in these new

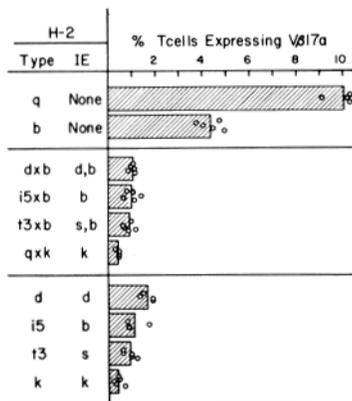


FIGURE 3. Elimination of Vβ17a⁺ T cells is dominant in IE⁺ × IE⁻ heterozygous mice. The extent of Vβ17a expression is shown for the Vβ^a homozygous F₂ and F₃ progeny of the crosses between IE⁺ and IE⁻ mice shown in Table II. The first panel contains the data for IE⁻, H-2 homozygous progeny; the second panel, for IE⁺, H-2 heterozygous mice; and the third panel, for IE⁺, H-2 homozygous mice. In two cases the heterozygous mice express the IE^b molecule as a result of complementation between Eα from the IE-expressing parent and Eβ^b from the non-IE-expressing C57L (H-2^b) parent. Each point represents a determination made in a different mouse. The data are expressed as the percent of total CD4⁺ plus CD8⁺ cells that expressed Vβ17a.

V β^a strains, where both the IE and V β 17a genes are homozygous, we could clearly see that not all IE molecules delete V β 17a⁺ T cells equally well (Table III, Fig. 4). Complementing our previous finding (10, 24) that V β 17a⁺ T cell hybridomas responded with lowest frequency to IE^d, the B10.D2 β L mice bearing IE^d had the greatest number of V β 17a⁺ T cells surviving tolerance. This was followed by B10.A(5R) β L(IE^b), B10.HTT β L(IE^s), and finally, B10.BR β BR(IE^k). In the last case the interpretation is clouded somewhat by a second deleting gene mapping to the K end of H-2^k (see below). It is worth noting that combination of the B10 background genes with those from C57BR to construct B10.BR β BR strain did not significantly alter the virtually complete deletion of V β 17a⁺ T cells.

In each of the IE⁺ strains, deletion of V β 17a⁺ T cells was evident among both the CD4⁺ and CD8⁺ T cells, although deletion among CD4⁺ T cells appeared more efficient (Table III, Fig. 2). For many CD4⁺/V β 17a⁺ T cell hybridomas, loss of or blockage of the CD4⁺ molecule results in loss of IE reactivity (data not shown). Thus, the deletion of CD8⁺/V β 17a⁺ T cells by IE suggests that deletion occurred at a stage of development when these cells bore CD4 and, therefore, reinforces the accumulating evidence for a CD4⁺/CD8⁺ intermediate in T cell development with at least some self-tolerance induction at this stage (25, 26). The skewing of V β 17a⁺ T cell deletion toward CD4⁺ T cells may suggest that, in addition, some of the deletion occurs after the cells have become CD4 or CD8 single-positive T cells, or could simply indicate that the small proportion of V β 17a⁺ T cells that are not IE reactive may be enriched in T cells selected by class I ligands in the thymus and are destined to become CD8⁺.

Differences in Expression of V β 17a in IE⁻ V β^a Mice. Some of the most noteworthy results on V β 17a expression were obtained in the new IE⁻ V β^a strains. For example, direct evidence that non-H-2 genes affect the levels of V β 17a⁺ T cells came from comparing the two V β^a H-2^a mice. There were considerably more V β 17a⁺ T cells in SWR mice than in B10.Q β BR mice, especially among CD4⁺ T cells. V β 17a expression was also considerably higher in SJL than in B10.S(7R), although the difference at H-2D as well as in the non-H-2 background genes may play a role here. On the other hand, V β 17a expression did not differ significantly among the three H-2^b mice carrying different mixes of the background genes from C57L and B10 mice, again pointing out the relatedness of the C57 mice.

One surprising result was the low expression of V β 17a⁺ T cells (1.3%) in IE⁻ B10.A(4R) β L mice (Table III). Comparison of these mice to any of the H-2^b V β^a mice suggests that a second H-2 gene mapping to the K end of H-2^k also causes significant deletion of V β 17a⁺ T cells. Alternatively, it is possible that the B10.A(4R) H-2 products simply poorly select V β 17a⁺ T cells during T cell development. Evi-

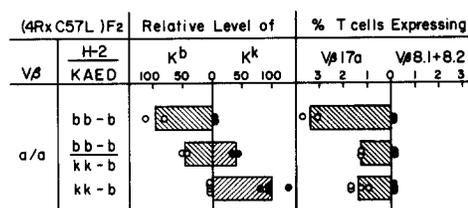


FIGURE 4. H-2 and V β typing of V β^a homozygous (B10.A(4R) \times C57L)F₂ mice. Eight V β^a homozygous mice were analyzed for H-2 and V β as in Fig. 1, except that 28-13-3 (anti-K^b) and 11-4.1 (anti-K^k) were used for the H-2 typing.

dence that this low expression is in fact due to deletion was seen during the analysis of the F₂ mice used to construct this strain (Fig. 4). Low expression of V β 17a was seen both among the mice homozygous and heterozygous for the H-2 type of B10.A(4R), reflecting the dominance expected of deletion due to self-tolerance. This non-IE-deleting elements perhaps explains why in H-2^k mice, which carry both this element and the IE^k molecule, the deletion of V β 17a⁺ T cells is virtually complete.

While the deleting *K* end gene product could be either IA^k or K^k, two observations favor K^k. First, although, as with IE, this deleting element deletes both CD4⁺ and CD8⁺ T cells in B10.A(4R) β _L mice, in this case the effect is more dramatic on CD8⁺ than CD4⁺ T cells. Second, in our studies of the reactivity of V β 17a⁺ T cell hybridomas (10, 24), we have found only occasional responsiveness to IA^k. At present, we are unable to study most cases of class I recognition in T cell hybridomas because the fusion partner, BW5147, supports CD4, but not CD8, expression in its hybrids (27). Therefore, it is likely in these hybridoma studies that a CD4-dependent reactivity to IA^k would have been found were it to exist, but a CD8-dependent response against K^k would have been missed.

With the exception of the B10.A(4R) β _L strain, expression of V β 17a among CD8⁺ T cells varied only by a factor of about two (3.6–7.3%). On the other hand there was a much greater variation among CD4⁺ T cells, with a low of 2.8% in C57L mice and a high of 18.5% in SWR. The high expression in B10.Q β _{BR} (13.2%) vs. low expression in the H-2^b mice (2.8–3.4%) established that much of this difference was due to H-2. This phenomenon could reflect another gene deleting V β 17a⁺ T cells in the H-2^b haplotype specific for CD4⁺ T cells; however, in this case, preliminary results have shown that high expression is dominant in (H-2^a \times H-2^b)F₁ mice (Blackman, M., P. Marrack, and J. Kappler, manuscript in preparation) favoring the view that in the thymus, IA^a is a much better selector of V β 17a⁺ T cells than is IA^b. We are currently testing this idea in the appropriate chimeric mice.

Discussion

Mice carrying the V β ^a haplotype should be useful in T cell repertoire studies for two reasons. First, they carry a functional structural gene for V β 17 (10, 11). Since receptors utilizing V β 17a have the unusual property of reacting with high frequency to B cell-presented IE molecules (10, 12, 28), V β ^a mice can be used to study the phenomenon of self-tolerance to class II MHC ligands. A possible class I ligand for V β 17a⁺ T cells in H-2^k may make the B10.A(4R) β _L strain useful in studying tolerance induction to class I MHC ligands. In addition, the dramatic difference in CD4⁺ V β 17a⁺ T cells between H-2^a and H-2^b suggests that IA^a selects V β 17a⁺ better than IA^b, perhaps offering a system in which the process of thymic positive selection can be studied.

A second feature of the V β ^a haplotype is the remarkable deletion in the middle of the complex of about half of the V β elements (9). Although one might suppose that this loss of receptor elements would be detrimental because it would limit the diversity of the $\alpha\beta$ receptor repertoire, the deletion has apparently been tolerated evolutionarily for some time (29). The immunological consequences of this deletion have not been extensively explored. We have suggested that this deletion and other genetic mechanisms that limit the expression of particular V β elements in different mice may play a role in the mouse population at large in balancing the advantage

of a large T cell repertoire against the potential involvement of these V β elements in autoimmune reactions (11, 12, 19, 30). The development of a set of V β ^a mice of similar background and carrying different IE⁺ and IE⁻ H-2 haplotypes should provide a useful tool to study this question.

Summary

A set of new mouse strains were produced that carry the V β ^a haplotype of the TCR- α/β and any of a number of different H-2 haplotypes on backgrounds derived from related C57BL, C57L, and C57BR mice. Study of V β 17a expression in these mice confirms the association between the presence of IE and the deletion of V β 17a⁺ T cells. A second H-2 gene causing deletion of V β 17a⁺ T cells was mapped in these mice to the K end of H-2^k, and H-2 influences on the level of selection of CD4⁺ V β 17a⁺ T cells were indicated.

We thank Janice White, Terri Wade, and Rhonda Richards for their excellent technical support.

Received for publication 16 November 1988 and in revised form 23 January 1989.

References

1. Chou, H., C. Nelson, S. Godambe, D. Chaplin, and D. Loh. 1987. Germline organization of the murine T cell receptor β -chain genes. *Science (Wash. DC)*. 238:545.
2. 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.
3. Lee, N., and M. Davis. 1988. T cell receptor β -chain genes in BW5147 and other AKR tumors. Deletion order of murine V β gene segments and possible 5' regulatory regions. *J. Immunol.* 140:1665.
4. Behlke, M., D. Spinella, H. Chou, W. Sha, D. Hartt, and D. Loh. 1985. T cell receptor β -chain expression: dependence on relatively few variable region genes. *Science (Wash. DC)*. 229:566.
5. Barth, R., S. Kim, N. Lan, T. Hunkapillar, N. Sobieck, A. Winoto, H. Gershenfeld, C. Okada, D. Hansburg, I. Weissman, and L. Hood. 1985. The murine T-cell receptor uses a limited repertoire of expressed V β gene segments. *Nature (Lond.)*. 316:517.
6. Patten, P., T. Yokota, J. Rothbard, Y. Chien, K. Arai, and M. Davis. 1984. Structure, expression and divergence of T-cell receptor β -chain variable regions. *Nature (Lond.)*. 312:40.
7. Singer, P., R. McEvilly, D. Noonan, F. Dixon, and A. Theofilopoulos. 1986. Clonal diversity and T-cell receptor β -chain variable gene expression in enlarged lymph nodes of MRL-lpr/lpr lupus mice. *Proc. Natl. Acad. Sci. USA*. 83:7018.
8. Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A. Schmitt-Verhulst, F. Fitch, L. Hood, and B. Malissen. 1986. Direct evidence for chromosomal inversion during T-cell receptor β -gene rearrangements. *Nature (Lond.)*. 319:28.
9. 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.
10. Kappler, J., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, R. Roehm, and P. Marrack. 1987. A T cell receptor V β segment that imparts reactivity to a class II major histocompatibility complex product. *Cell*. 49:263.
11. Wade, T., J. Bill, P. Marrack, P. C. Palmer, and J. Kappler. 1988. Molecular basis for the non-expression of V β 17 in some strains of mice. *J. Immunol.* 141:2165.
12. Kappler, J., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. *Cell*. 49:273.

13. Oi, V., P. Jones, J. Goding, L. Herzenberg, and L. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81:115.
14. Ozato, K., N. Mayer, and D. Sachs. 1982. Monoclonal antibodies to MHC antigens. IV. A series of hybrid clones producing anti-H-2^d antibodies and an estimation of expression of H-2^d antigens on the surface of these cells. *Transplantation (Baltimore)*. 34:113.
15. Ozato, K., and D. H. Sachs. 1981. Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2^b haplotype reveal genetic control of isotype expression. *J. Immunol.* 126:317.
16. Dialynas, D., Z. Quan, K. Wall, A. Pierres, J. Quintans, M. Loken, M. Pierres, and F. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK-1.5: similarity of L3T4 to the human Leu 3/T4 molecule and the possible involvement of L3T4 in Class II MHC antigen reactivity. *J. Immunol.* 131:2445.
17. Ledbetter, J., and L. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse differentiation antigens. *Immunol. Rev.* 47:63.
18. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. 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.
19. Kappler, J. W., U. Staerz, J. White, and P. C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature (Lond.)*. 332:35.
20. Gey, G., and M. Gey. 1936. The maintenance of human normal and tumor cells in continuous culture. I. Preliminary report: cultivation of mesoblastic tumors and normal tissue and notes on methods of cultivation. *Am. J. Cancer.* 27:45.
21. Julius, M., E. Simpson, and L. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived lymphocytes. *Eur. J. Immunol.* 3:645.
22. Kappler, J., and P. Marrack. 1977. The role of H-2-linked genes in helper T cell function. I. In vitro expression in B cells of immune response genes controlling helper T cell activity. *J. Exp. Med.* 146:1748.
23. Roehm, N., A. Carbone, E. Kushnir, B. Taylor, R. Riblet, P. Marrack, and J. Kappler. 1985. The major histocompatibility complex-restricted antigen receptor on T cells: the genetics of expression of an allotype. *J. Immunol.* 135:2176.
24. Blackman, M. A., J. W. Kappler, and P. Marrack. 1988. T-cell specificity and repertoire. *Immunol. Rev.* 101:5.
25. Kisielow, P., H. Bluthmann, U. D. Staerz, M. Steinmetz, and H. vonBoehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4⁺8⁺ thymocytes. *Nature (Lond.)*. 333:742.
26. Sha, W., C. Nelson, R. Newberry, D. Kranz, J. Russell, and D. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature (Lond.)*. 336:73.
27. Carbone, A. M., P. Marrack, and J. Kappler. 1988. Remethylation at sites 5' of the murine Lyt 2 gene in association with shut down of Lyt 2 expression. *J. Immunol.* 333:1369.
28. Marrack, P., and J. Kappler. 1988. T cells can distinguish between allogeneic major histocompatibility complex products or difference cell types. *Nature (Lond.)*. 332:6167.
29. Huppi, K., B. D'Hoostelaere, B. Mock, E. Jouvin-Marche, M. Behlke, H. Chou, R. Berry, and D. Loh. 1988. T-cell receptor VT β genes in natural populations of mice. *Immunogenetics.* 27:51.
30. 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.