

SELECTIVE DECREASES IN T CELL RECEPTOR V β EXPRESSION

Decreased Expression of Specific V β Families Is Associated with Expression of Multiple MHC and Non-MHC Gene Products

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Since the discovery of the structure of the TCR (reviewed in reference 1), many studies have been directed toward elucidating the role of specific V region gene products in determining antigen specificity. The role of V β has been most widely examined in these studies. Analysis of T cell clones and/or T cell hybridomas of defined antigen specificity has in some instances revealed no correlation between the V β genes used and antigen specificity (2, 3). Other studies have shown strong correlations, such as the preferential usage of V β 3 and V α 11 by T cells specific for cytochrome C in association with E α E β (4). T cells recognizing self MHC plus foreign protein antigen are generally present in the T cell repertoire at low precursor frequencies. In contrast, T cells specific for minor lymphocyte stimulatory (Mls)¹ antigens or for allogeneic MHC determinants have been shown to occur at uniquely high precursor frequencies. Analysis of TCR usage in T cells specific for Mls and MHC determinants has revealed several striking correlations of V β expression with T cell specificity. Characterization of T cell hybridomas that are alloreactive to E α E β class II MHC products revealed a strong correlation between V β 17a expression and this specificity (5). Work by several groups has also demonstrated strong associations between V β usage and Mls reactivity. MacDonald et al. (6) and Kappler et al. (7) demonstrated the Mls^a reactivity correlated with V β 6 and V β 8.1 usage, respectively. More recently, it has been demonstrated that recognition of Mls^c is associated with V β 3 usage in T cell clones or hybridomas (8-10). Thus, at least in the instances of alloreactivity to MHC and Mls determinants, there exists a strong correlation between particular V β usage and T cell antigen specificity. Moreover, it appears that in these instances the expression of an appropriate V β segment is sufficient to confer specificity independent of other β chain or α chain segments.

It has been suggested that T cells undergo both positive and negative selection in the thymus, presumably after rearrangement and expression of a functional TCR. In particular, it has been postulated that negative selection during thymic education represents a process by which self-reactive T cells are deleted from the repertoire. The work by Kappler et al. (11) studying V β 17a expression by T cells appears to

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¹ Abbreviation used in this paper: Mls, minor lymphocyte stimulatory.

represent a demonstration of clonal deletion in the thymus of mice that express an E α E β product. Similar depletion of V β 11 expression has been reported in E α E β -expressing strains (12, 13). Clonal deletion of self-reactive T cells as a mechanism of self tolerance can also be shown in mice expressing Mls^a (deletion of V β 6⁺ and V β 8.1⁺ T cells) (6, 7) or Mls^c (deletion of V β 3⁺ T cells) (8, 10).

Previous reports of TCR V β usage have either studied expression of a single V β in a wide panel of strains (6, 7, 10, 12, 13) or expression of multiple V β s in a limited strain distribution (14, 15) and have identified instances of clonal deletion of potentially autoreactive T cells specific for either self E α E β or Mls antigens. These studies raise the question of how frequent such deletions of T cells expressing specific V β regions may be, and of whether such deletions occur as a result of reactivity to a limited or more extensive set of self determinants. To address this, we have analyzed expression of 16 V β gene products in 30 different strains of mice. Results of the present study demonstrate that strain-specific decreases in mRNA expression occur for at least 8 of the 16 V β s analyzed, and that expression of MHC, Mls^a, and Mls^c gene products play a dominant role in this effect.

Materials and Methods

Mice. C57BL6/NCR, CB6F1, A/J, C3H/HeJ, and DBA/2NCR mice were obtained from the Frederick Cancer Research Facility (Frederick, MD). BALB.B, BALB.K, BALB/ckh, (B10.A \times A/Sx)F₁, B10.M, B10.Q, B10.S, B10.RIII, B10.A(2R), B10.A(3R), and B10.A(4R) mice were generous gifts from Dr. David Sachs (NIH). (B10.BR \times AKR/J)F₁ were bred at Bioqual (Rockville, MD). All other mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Preparation of T Cell RNA. Single cell suspensions from a pool of five spleens were passed over rabbit anti-mouse Ig-coated plates to enrich for T cells. The resulting nonadherent T cells were then cultured at a concentration of 2×10^6 /ml for 48–72 h with 2 μ g/ml of Con A, harvested, and RNA was extracted by a cesium chloride/guanidinium method followed by phenol-chloroform extraction and precipitation (16).

Northern Blot Analysis. Northern analysis was carried out as previously described (9), except that labeling of probes was performed by random primer extension, and 20 μ g of RNA was loaded per lane. V β -specific probes were a generous gift from Dr. Dennis Loh (Washington University, St. Louis, MO). The C β probe was obtained from Dr. Stephen Hedrick (University of California, San Diego, CA). Individual filters were hybridized sequentially to multiple V β probes by stripping and rehybridization and were then hybridized to the C β -specific probe.

Densitometry was performed on a scanning densitometer (1650; Bio-Rad Laboratories, Richmond, CA). V β expression for each strain was standardized to C β expression and values were expressed in relation to those obtained for the B6 strain. Replicate experiments were performed in which independent gels and hybridizations were carried out to test the reproducibility of findings, and densitometric data are presented as mean values of replicate determinations.

Results

To evaluate the influence of both MHC and non-MHC haplotypes on the V β gene products expressed, RNA was isolated from Con A T cell blasts from 30 different strains of mice and was analyzed for V β gene usage with 16 V β -specific probes; C β expression was determined as a means of quantitating total β chain mRNA. Included in this panel were animals of 10 different MHC haplotypes and 11 different non-MHC backgrounds, as well as seven different F₁ combinations. Activation of T cells with Con A resulted in a shift in the CD4⁺/CD8⁺ ratio from \sim 2 in unacti-

vated T cells to 0.5 in Con A-activated T cells. However, flow cytometric analysis of serologically detectable Vβ8 and Vβ6 expression revealed that Con A stimulation did not alter the percentage of T cells expressing specific Vβ gene products (data not shown).

Expression of Vβ5, -11, -12, and -16 Is Influenced by MHC Genes. Northern blot analysis of RNA expression of Vβ11, -12, and -16 showed substantially higher levels of each of these in H-2^b mice when compared with MHC congenic H-2^a, H-2^k, or H-2^d animals on each of four different backgrounds examined (Fig. 1, Table I). The same pattern was seen with Vβ5 expression, although the quantitative differences in expression between H-2^b and other strains were frequently not so great as the differences observed for Vβ11, -12, and -16 (Table I). This may be related to the fact that Vβ5 represents a multigene family, in contrast to these other Vβs, and that deletion of T cells expressing one of the two Vβ5 members may occur. The quantitatively limited strain differences in Vβ5 expression precluded further evaluation of this Vβ regulation.

While there appeared to be overall consistency in the decreased expression of Vβ5, -11, -12 and -16 in H-2^a, H-2^k, or H-2^d strains, there was one strain, C58/J, that differed in its pattern of expression of these Vβs. This strain, which is H-2^k, showed decreased expression of Vβ5 and Vβ16, but not Vβ11 and Vβ12 (Fig. 1, Table I). One potential interpretation of this result is that a polymorphism exists in the MHC element influencing expression of these Vβs in C58/J compared with other H-2^k

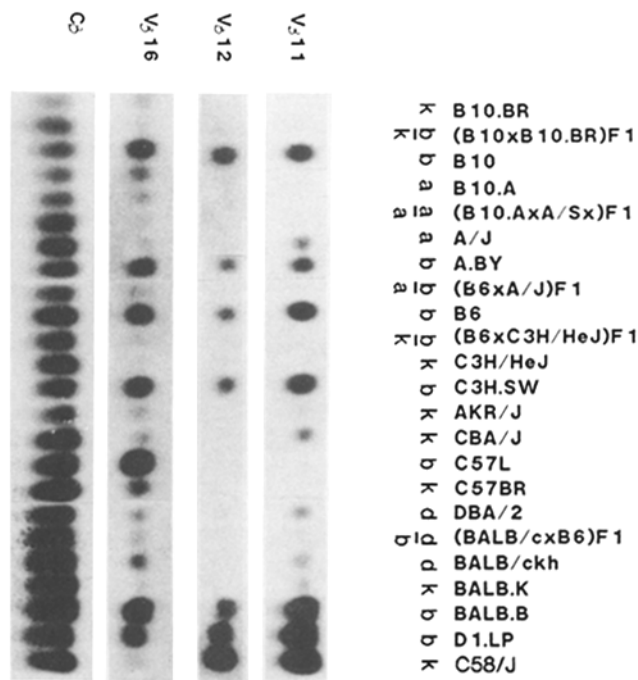


FIGURE 1. Expression of Vβ11, -12, and -16 is influenced by MHC gene products. (A) A Northern blot of RNA isolated from Con A-activated T cells from various inbred strains was hybridized sequentially with V region-specific probes, Vβ11, Vβ12, Vβ16, and finally with a Cβ-specific probe. Absence of expression of Vβ11 and -12 in C57L and C57BR strains is due to a genomic deletion in these strains (21).

TABLE I
Effect of MHC on TCR V β Expression

	H-2	Relative V β expression			
		V β 5	V β 11	V β 12	V β 16
B6	b	1.00	1.00	1.00	1.00
B10.BR	k	0.42	0.14	0.23	0.49
(B10 \times B10.BR)F ₁	b/k	0.53	0.15	0.04	0.15
B10	b	1.29	0.86	1.43	1.31
B10.A	a	0.36	0.01	0.20	0.33
A/J	a	0.26	0.47	0.18	0.14
A.BY	b	0.67	0.72	0.92	1.09
(B6 \times A/J)F ₁	b/a	0.25	0.16	0.13	0.31
C3H/HeJ	k	0.39	0.08	0.13	0.12
C3H, SW	b	0.91	1.37	0.96	1.22
(B6 \times C3H/HeJ)F ₁	b/k	0.28	0.10	0.15	0.15
BALB/c	d	0.41	0.15	0.13	0.36
BALB.K	k	0.59	0.11	0.05	0.14
BALB.B	b	0.84	0.77	0.55	1.02
(BALB/c \times B6)F ₁	b/d	0.42	0.13	0.15	0.21
C58/J	k	0.29	1.19	1.49	0.15

Values are expressed as the means of densitometric readings on two to four individual filters. Values for V β 11 are densitometric readings on a single filter.

strains. Alternatively, differential expression may be due to background differences between C58/J and other H-2^k strains in which products of genes outside the H-2 region could influence V β expression in the context of MHC gene products, as has been previously suggested in the case of V β 11 expression (12).

The mechanism underlying the observed differences in specific V β expression could involve positive selection for these V β s in H-2^b strains, or alternatively, negative selection in the other H-2 genotypes (5). To determine whether positive or negative selection is involved, RNA was isolated from F₁ mice resulting from crosses between H-2^b and other H-2 haplotypes. If positive selection is involved, one would expect high V β expression in F₁ mice, whereas negative selection would result in V β deletion in these F₁ animals. As can be seen in Fig. 1 and Table I, the F₁s between H-2^b and H-2^a ([B10 \times B10.A]F₁ and B6AF₁), H-2^k ([B10 \times B10.BR]F₁ and B6C3F₁), or H-2^d(CB6F₁) had decreased expression of V β 5, -11, -12, and -16, comparable with the levels seen in the parental H-2^a, H-2^d, or H-2^k strains. These findings indicate that negative selection is involved in the MHC-related decreased expression of each of these four V β segments. In the case of V β 11 expression, it was noted both in this study, as well as previously by mAb staining (12), that A background strains showed less dramatic decreases in expression than strains of similar H-2. Interestingly, F₁ animals between C57BL and A backgrounds showed more dramatic decreases in V β 11 expression than did the A background parent, consistent with a role of non-MHC products expressed by C57BL but not A strains in V β 11 expression.

One of the many differences between H-2^b mice and H-2^a, H-2^k, or H-2^d strains is the lack of expression of an E α E β product in H-2^b strains. As in the case of V β 17a, which is not expressed in E α E β ⁺ strains, it was of interest to determine whether E α E β expression is the controlling factor in these cases as well. RNA was isolated

from Con A-activated T cells generated from a series of B10 congenic strains that either do or do not express $E_{\alpha}E_{\beta}$. As seen in Table II, expression of $V\beta 11$ and -16 was high in B10, B10.M, B10.S, and B10.Q, which lack $E_{\alpha}E_{\beta}$ expression, but was decreased in B10.A and B10.RIII, which express $E_{\alpha}E_{\beta}$. The relationship of $V\beta 12$ to $E_{\alpha}E_{\beta}$ expression was less clear in this series of MHC congenic strains. Further analysis was carried out with the intra-MHC recombinant strains B10.A(2R), B10.A(3R), and B10.A(4R). In particular, the strains B10.A(2R) and B10.A(4R) appear to differ only in their expression and nonexpression, respectively, of an $E_{\alpha}E_{\beta}$ product. Expression of $V\beta 11$ and -16 was greater in 4R than in 2R, indicating that the presence of an $E_{\alpha}E_{\beta}$ product results in reduced expression of these $V\beta$ gene products. However, these findings do not preclude effects by other MHC gene products, particularly since the decrease in $V\beta 16$ expression is not as dramatic in 2R and 3R as in B10.A. $V\beta 12$ expression, on the other hand, was comparably low in all three recombinant strains, suggesting the existence of multiple MHC influences, at least some of which are not related to $E_{\alpha}E_{\beta}$ expression. Taken together, these data indicate that the patterns of decreased expression of $V\beta 11$, -12 , and -16 are distinct from one another (Table I and II), and that the ligands responsible for the deletion of T cells expressing each of these $V\beta$ s are therefore presumably distinct.

Expression of $V\beta 3$ Is Eliminated in Mls^c Strains. $V\beta 3$ usage by T cells has previously been correlated with recognition of the Mls^c gene product, and Mls^c strains have been shown to have reduced expression of $V\beta 3$ (8, 10). Probing for $V\beta 3$ mRNA in the present study revealed that strains of mice that are Mls^a or Mls^b showed high expression, whereas Mls^c strains showed substantial depletion of $V\beta 3$ mRNA (Fig. 2, Table III). These decreases in expression were also influenced by the MHC type of the Mls^c strain, with $V\beta 3$ depletions occurring in H-2^a, H-2^k, or H-2^d Mls^c strains, but not in H-2^b Mls^c strains. This pattern of $V\beta 3$ expression correlates well with the reported MHC influence on T cell stimulation by Mls , in that H-2^b strains that are genotypically Mls^c are generally poor stimulators or nonstimulators of an Mls^c -specific response (17). One exception to this generalization in the present study was A.BY, a strain that is H-2^b and Mls^c but revealed little or no $V\beta 3$ expression, despite the fact that this strain is a poor stimulator for Mls^c responses (17). A more extensive analysis of the relationship between decreased expression of $V\beta 3$ and Mls^c

TABLE II
V β Expression in B10 Congenic Strains

	I-E	Relative $V\beta$ expression		
		$V\beta 11$	$V\beta 12$	$V\beta 16$
B10	-	1.00	1.00	1.00
B10.A	+	0.10	0.19	0.21
B10.M	-	1.56	0.67	1.44
B10.S	-	1.77	0.91	1.22
B10.RIII	+	0.05	0.34	0.33
B10.Q	-	0.87	0.54	1.20
B10.A(2R)	+	0.33	0.29	0.63
B10.A(3R)	+	0.24	0.18	0.48
B10.A(4R)	-	0.93	0.37	1.10

Values are expressed as the means of densitometric readings of two to four individual filters.

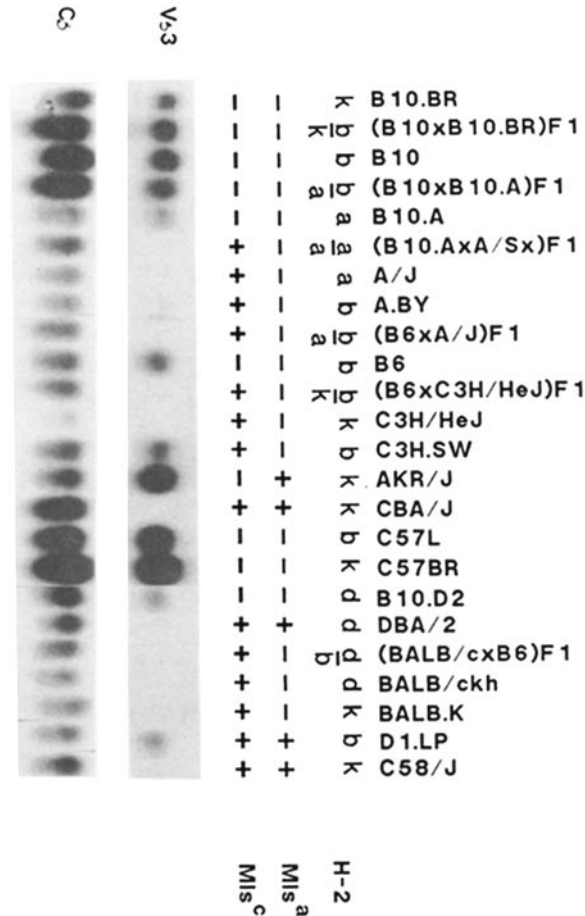


FIGURE 2. Expression of V β 3 is absent in Mls^{c+} strains of mice. A Northern blot of RNA isolated from Con A-activated T cells from various inbred strains was probed for expression of V β 3, then C β .

expression is now in progress. High levels of V β 3 expression occurred in the strain AKR/J relative to expression in B6. Potential mechanisms for this increase are discussed below.

The lack of expression of V β 3 message in Mls^c mice has been reported to be due to negative selection (8, 10). Consistent with this interpretation, F₁ mice between Mls^c and Mls^b lacked expression of V β 3 message in the present study. (BALB/c × B6)F₁ (H-2^{d/b}, Mls^{c/b}), (B10.A × A/Sx)F₁ (H-2^{a/a}, Mls^{b/c}), B6AF₁ (H-2^{b/a}, Mls^{b/c}), and B6C3F₁ (H-2^{b/k}, Mls^{b/c}) showed decreased V β 3 expression, whereas (B10 × B10.A)F₁ (H-2^{b/a}, Mls^{b/b}), (B10 × B10.BR)F₁, (H-2^{b/k}, Mls^{b/b}), and (B10.BR × AKR/J)F₁ (H-2^{k/k}, Mls^{b/a}) expressed relatively high levels of V β 3 (Table III).

Expression of V β 6 and V β 9 Is Decreased in Mls^a Strains. Expression of V β 6 had previously been demonstrated to be negatively effected by expression of Mls^a (6). In this study, levels of both V β 6 and V β 9 correlated in the panel of strains tested with Mls^a type (Fig. 2, Table III). There was a significant decrease in hybridization of the V β 6- and V β 9-specific probes in 5/5 Mls^{a+} strains compared with levels in Mls^{a-}-nonexpressing mice expressing strains. Decreased expression of both of these V β s

TABLE III
Effect of MIs on TCR V β Expression

	MIs	H-2	Relative V β expression			
			V β 3	V β 6	V β 9	V β 7
B6	b	b	1.00	1.00	1.00	1.00
(B6 \times A/J)F ₁	b/c	b/a	0.05	1.39	0.73	0.56
A/J	c	a	0.03	2.25	1.06	0.39
A.BY	c	b	0.09	1.74	0.66	1.01
C3H/HeJ	c	k	0.08	3.33	0.75	0.13
C3H.SW	c	b	0.94	2.73	0.98	0.66
(B6 \times C3H/HeJ)F ₁	b/c	b/k	0.04	1.72	0.62	0.47
AKR/J	a	k	5.23	0.07	0.05	0.32
B10.BR	b	k	2.15	1.15	1.27	0.82
(B10.BR \times AKR/J)F ₁	b/a	k/k	1.94	0.05	0.14	0.22
BALB/c	c	d	0.13	2.50	0.94	2.07
BALB.B	c	b	0.70	1.52	0.74	0.75
BALB.K	c	k	0.15	3.35	0.78	0.45
(BALB/c \times B6)F ₁	c/b	d/b	0.13	2.51	1.13	0.17
CBA/J	a,c	k	0.12	0.04	0.12	0.38
DBA/2	a,c	d	0.14	0.11	0.08	0.16
C58/J	a,c	k	0.08	0.06	0.21	0.12
D1.LP	a,c	b	0.60	0.14	0.28	0.80

Values are expressed as the means of densitometric readings of two to three individual filters.

appears to be a result of negative selection in that decreased expression is dominant in the MIs^a \times MIs^b F₁, B10.BR \times AKR/J (Table III). The previously described deletion of V β 8.1 (but not V β 8.2 or V β 8.3)-expressing cells (7) was not reflected in detectable decreases in overall V β 8 mRNA levels (data not shown). This is probably attributable to the difficulty in detecting changes in only one of the three members of the V β 8 family. Increased expression of V β 6 occurred in some strains, for example BALB/c and BALB.K, relative to expression in B6. Potential mechanisms responsible for such increases are discussed later.

Expression of V β 7 Is Influenced by both MHC and non-MHC Genes. V β 7 expression also differed substantially among the strains examined. In this instance, the genetic influences on V β expression appear to be complex and to include effects of both MHC and non-MHC genes. MHC influences were suggested by decreased expression of V β 7 in C3H/HeJ (H-2^k) vs. MHC congenic C3H.SW (H-2^b) mice and by similar differences among other MHC congenic combinations (Table III). In addition, non-MHC differences were apparent, for example, in the high expression of V β 7 by the H-2^k strain B10.BR and the lower expression in H-2^k strains C3H/HeJ, AKR/J, BALB.K, CBA/J, and C58/J. The identity of the non-MHC gene products influencing V β 7 is not clear, although an influence of both MIs^a and MIs^c in association with H-2^k products is consistent with the observed strain distribution. Further evidence for a role of multiple genes in decreased V β 7 expression was provided by the observation of genetic complementation in the CB6F₁ (BALB/c \times B6)F₁, which showed a decrease in V β 7 relative to both parental strains that expressed high

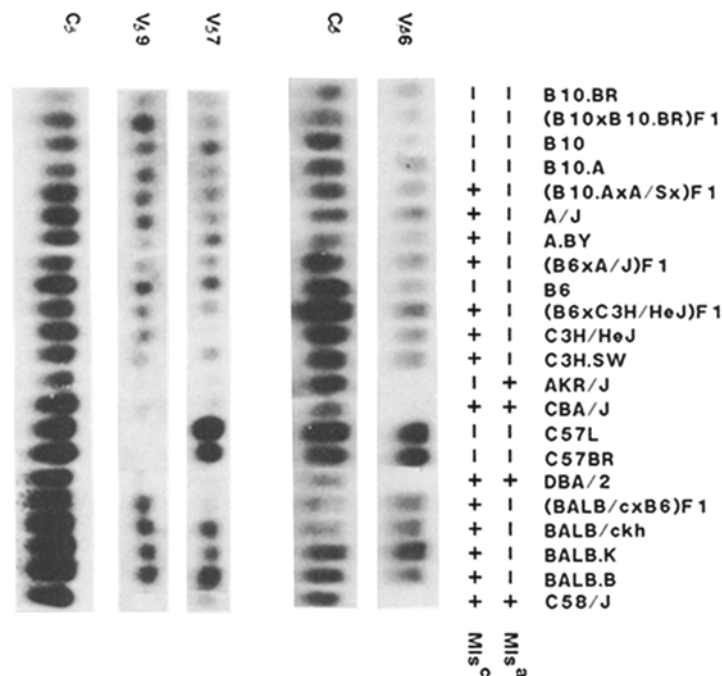


FIGURE 3. Expression of V β 6, -7, and -9 is decreased in MIs^{a+} strains of mice. (A) A Northern blot of RNA isolated from Con A-activated T cells from various inbred strains was hybridized first with a V β 6-specific probe, then with a C β -specific probe. (B) A Northern blot of RNA isolated from Con A-activated T cells from various inbred strains was hybridized sequentially with V region-specific probes, V β 7 and V β 9, then finally with a C β -specific probe. Absence of expression of V β 9 in C57L and C57BR strains is due to a genomic deletion in these strains (21).

levels. Further genetic analysis will be necessary to discern what factors influence V β 7 expression in these strains.

Expression of Several V β Gene Products Is not Selectively Decreased in any Strain Analyzed. Expression of V β 1, -4, -10, -13, -14, and -15 was not significantly decreased in any strains tested relative to the arbitrarily selected reference strain B6. Interestingly, relatively increased expression of some V β s was seen in certain strains, in particular in strains that exhibit significantly decreased expression of several other V β families as a result of negative selection. The findings presented in this study indicate that substantial decreases in expression of V β 3, -5, -7, -9, -11, -12, and -16 occur in strains expressing appropriate self determinants. It would be expected that strains with decreased expression in most or all of these V β s (e.g., CBA/J or AKR/J) would show compensatory increases in expression of other V β gene products. In fact, both CBA/J and AKR/J showed substantial increases in expression of several V β s, such as V β 1 and V β 2 relative to B6 (Table IV). In addition, AKR/J (H-2^k, MIs^a) showed increased expression of V β 3, deletion of which is associated with expression of MIs^c. The strains C57L and C57BR, which have deleted 6/17 V β families at the genomic level, would be expected to show similar compensatory increases in the remaining V β s; increases in expression were seen in several V β s in C57L and in C57BR relative to B6 (Table IV). If these increases are due to a compensatory effect resulting

TABLE IV
Strain-specific Increases in V β Expression

	H-2	Mls	Relative V β expression		
			V β 1	V β 2	V β 3
B6	b	b	1.00	1.00	1.00
C57L	b	b	0.88	2.85	4.88
C57BR	k	b	1.72	3.38	6.46
CBA/J	k	a,c	7.56	2.65	0.12
AKR/J	k	a	3.39	2.59	5.23

Values are expressed as the means of densitometric readings of two individual filters.

from decreased expression of multiple other V β s, it is unclear why these differences occur in some but not all of the V β s for a particular strain (e.g., why V β 1 expression is not increased in C57L and C57BR). In some instances, as yet undefined negative selection may also influence overall expression of these V β s. An alternative explanation of the selective increases is positive selection. This is not excluded in the present study but appears to be less likely since the selective increases in V β expression were seen predominantly in strains of mice that have decreased expression of multiple other V β s in association with the expression of multiple MHC and non-MHC self determinants.

Discussion

Previous reports of TCR V β usage, studying either expression of a single V β in a wide panel of strains (6, 7, 10, 12, 13), or expression of multiple V β s in a limited strain distribution (14, 15), have identified instances of clonal deletion of potentially autoreactive T cells specific for either self E α E β or Mls antigens. It was therefore of interest to determine (a) the extent of such deletions in the V β repertoire; and (b) the full range of self antigens that can exert such influences on V β usage. To pursue this question, RNA from 30 strains of mice was analyzed with 16 V β -specific probes to determine whether differential V β usage could be detected in strains of mice of various MHC or non-MHC haplotypes. The results of this survey revealed apparently dominant negative influences of Mls^a, Mls^c, and MHC expression on the T cell V β repertoire. It was shown that expression of V β 5, -11, -12, and -16 is influenced by MHC gene products. The patterns of decreased expression observed in intra-MHC recombinant strains, as well as in strains of different non-MHC backgrounds, were different for V β 11, -12, and -16, suggesting that distinct MHC and/or non-MHC ligands are responsible for the deletion of T cells expressing these three V β segments. The presence of an E α E β product was previously reported to result in decreased V β 17a (11) and V β 11 (12, 13) expression. The present study confirmed this effect on V β 11 and also demonstrated a similar influence on V β 16 mRNA expression. Potentially more complex influences of MHC on V β 5 and V β 12 expression were observed that appear to include influences of MHC products other than E α E β . Results presented in this report are consistent with and extend recent data by Bill et al. that demonstrated increased usage of V β 5, -11, and -12 in T cell hybridomas derived from MHC congenics B10 and B10.Q relative to those derived from

B10.BR (15). However, the observed influence of MHC on V β 12 expression is in contrast to a recent report by Okada and Weissman (14) that attributed decreased expression of V β 12 to an influence of Mls^a. In the present study, V β 12 expression was high in D1.LP, an H-2^b Mls^a strain (Fig. 1) and was low in several Mls^{a-} strains. The suggestion by Okada and Weissman that Mls^a expression resulted in diminished expression of V β 4 was not confirmed by more extensive strain analysis used in the present study (data not shown). Expression of Mls^a does appear to result in decreases not only of V β 6 (6) and 8.1 (7) expression, as previously reported, but also of V β 9. Expression of Mls^c was associated with decreased V β 3 expression in a wide panel of strains, confirming earlier reports (8, 10). V β 7 expression appears to be influenced by both MHC and non-MHC gene products that are yet undefined.

It has been proposed that T cells undergo both positive and negative selection during thymic education in which T cells are first positively selected for affinity to self MHC gene products, and subsequently, those cells with high affinity for self MHC are eliminated to prevent autoreactivity. It is interesting that in the several cases analyzed to date, V β expression appears to be negatively regulated, as seen in the analysis of F₁ mice (6-8, 10, 11, 13). The results of the present study are consistent with the interpretation that decreased expression of V β 3, -5, -6, -9, -11, -12, and -16 are all based upon negative selection, since each of these effects were found to be dominant in F₁ mice. Apparent examples of positive selection have been reported for V β 6 by MacDonald et al. (18), and for expression of a transgenic $\alpha\beta$ receptor by Kisielow et al. (19). It cannot be concluded with certainty whether the increases noted in the present study in V β expression in several strains are due to positive selection, or are simply compensatory increases in those strains with significantly decreased numbers of V β families expressed.

The present study has demonstrated that decreases in specific TCR V β expression are indeed extensive. In searching to identify the spectrum of self antigens that may function to mold the TCR V β repertoire, major effects were detected for the non-MHC antigens, Mls^a and Mls^c, and for the MHC product, E α E β . A role of MHC products other than E α E β is indicated in the present study for decreased expression of V β 12 in particular. In addition, a possible role of non-MHC, non-Mls products is indicated for V β 11, -12, and -16 expression as exemplified in the behavior of the strain C58/J in the present study, for V β 11 (12, 13) and V β 17a (20) as recently described, and potentially for V β 7 expression as well. It is interesting that strains of mice expressing all three of the determinants Mls^a, Mls^c, and E α E β , such as CBA/J and DBA/2, have decreased expression in 9 of 16 available V β families with no deficiencies yet detected in their ability to respond to foreign antigen. This is not unlike the case in which a genomic deletion has resulted in the loss of six V β families in the strains C57L, C57BR, and SJL (21). The ability to respond to foreign antigen in the absence of numerous V β s suggests a substantial plasticity in the TCR V β repertoire.

Products of the E α E β , Mls^a, and Mls^c gene loci are expressed by some but not all inbred strains of mice so that the corresponding V β depletions similarly occur in some but not all members of the species. It is possible that while not essential for survival, expression of E α E β and Mls determinants provides some selective advantage to the animal. In addition to the direct advantage that might be provided by expression of these determinants as functional cell surface molecules, the associated

V β deletions might also be advantageous, for example, in circumstances in which a given TCR-mediated response is a threat to survival. Such circumstances would include autoimmune responses or the potentially negative consequences of specific TCR V β interactions with microbial products (22, 23) as suggested by White et al. (22).

Summary

Previous reports of TCR V β usage, studying either expression of a single V β in a wide panel of strains (6, 7, 10, 12, 13), or expression of multiple V β s in a very limited strain distribution (14, 15), have identified instances of clonal deletion of potentially autoreactive T cells specific for either self E α E β or minor lymphocyte stimulatory (Mls) antigens. The present study has investigated the range of self antigens that can influence V β usage by evaluating expression of 16 V β families in 30 strains of mice. It was found that significant decreases in expression occur in at least 8 of the 16 V β families and that dominant influences on the T cell V β repertoire are exerted by expression of Mls^a, Mls^c, and MHC gene products. Decreased expressions of V β 5, -11, -12, and -16 were influenced by MHC gene products. The patterns of decreased expression seen in intra-MHC recombinant strains and strains of different non-MHC background were distinct for V β 11, -12, and -16, suggesting that different ligands are involved in the deletion of T cells expressing each of these V β genes. Mice expressing Mls^a show decreased expression of V β 9 as well as V β 6. Mls^c mice lacked V β 3 expression in those strains where the expressed MHC type was compatible with a strongly stimulatory Mls^c phenotype. V β 7 was strongly influenced by both MHC and non-MHC products that are not yet identified. These results demonstrate that strain-specific decreases of mRNA expression occur in a major portion of the TCR repertoire. Self antigens including Mls^a, Mls^c, and E α E β , as well as additional MHC and non-MHC products, appear to induce these decreases in expression in the process of eliminating self-reactive T cells from the mature T cell pool.

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