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An analysis of T-cell receptor variable region gene expression in major histocompatibility complex disparate mice

(T-cell repertoire/major histocompatibility complex restriction)

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ABSTRACT **To define the impact of major histocompatibility complex (MHC)-encoded glycoproteins on the selection of the T-cell receptor repertoire, we have determined the frequency with which T-cell receptor variable region (V_α and V_β) genes are expressed in T cells from MHC disparate mice. Approximately 500 T-cell hybridomas were generated from each of three strains of MHC congenic mice [B10 ($H\text{-}2^b$), B10.BR ($H\text{-}2^k$), and B10.Q ($H\text{-}2^q$)] by fusing mitogen-stimulated lymph node T cells with the thymoma BW5147. RNA was prepared from 1629 individual hybridomas and analyzed for the expression of 10 V_α and 16 V_β gene families. These experiments reveal significant differences in the relative contributions of 1 V_α gene family ($V_\alpha 3$) and several V_β gene segments ($V_\beta 5.1$, -5.2, -11, and -12) to the T-cell receptor repertoire of MHC disparate mice.**

The antigen receptor on T lymphocytes is a 90-kDa heterodimer comprised of an α- and a β-chain (1). These chains are encoded by somatically rearranging variable (V), diversity (D), and joining (J) region gene segments (2–6). The ligand for this class of receptors is a degraded protein fragment bound to a glycoprotein encoded by the major histocompatibility complex (MHC) (7–9); this explains in part the phenomenon of MHC restriction of T-cell antigen recognition described 14 years ago by Zinkernagel and Doherty (10). A second class of T-lymphocyte receptors, comprised of γ- and δ-chains, is present on developing thymocytes and on a small fraction of peripheral T cells (11–14); however, their function and their relation to α/β T-cell receptors (TCRs) is unclear. The MHC requirement for antigen recognition by α/β T cells is not generic but is allele specific in that TCRs recognize a specific allelic form of a MHC glycoprotein on a presenting or target cell (10). The MHC allele specificity of T-cell recognition is generated somatically in the thymus and is presumably the result of positive clonal selection that occurs during T-cell ontogeny (15, 16). Thymocytes are subjected to negative selection pressures as well. Kappler *et al.* have determined that a set of T cells whose antigen receptors share a V_β domain (V_β17a) are clonally deleted in mouse strains expressing an I-E molecule (17, 18). Thus, the repertoire of TCRs is shaped by both positive and negative selective influences.

Collectively, these experiments predict that the population of TCRs (i.e., the TCR repertoire) will differ among mice expressing different alleles of the MHC. To examine this point in detail, we have generated large panels of random T-cell hybridomas from three MHC disparate (i.e., MHC congenic) strains of mice and determined the frequency with which V_α and V_β gene segments are expressed in these hybrids. These experiments indicate that the frequency of expression of several V_β and V_α gene segments among mature peripheral T cells is influenced by the animal's MHC genotype.

MATERIALS AND METHODS

Generation of T-Cell Hybridomas. Lymph node cells from individual mice [four B10 ($H\text{-}2^b$), three B10.BR ($H\text{-}2^k$), and five B10.Q ($H\text{-}2^q$)] were separately cultured for 2 days in the presence of Con A (3 μg/ml) and separately fused to the thymoma BW5147 by standard methods (19). All fusions were plated at a low density such that >90% of the hybridomas were clonal. Hybridomas expressing >2 V_α or >2 V_β genes were assumed to be nonclonal and were not considered into the data base. These fusions generated 555, 583, and 491 hybrids from B10, B10.BR, and B10.Q Con A blasts, respectively. Cells (10^7) from each hybrid were grown, washed in balanced salt solution, and frozen for RNA extraction at a later time.

RNA Extraction and Hybridization Analyses. RNA was isolated by a slight modification of the protocol described by Cheley and Anderson (20); instead of passage through a 22-g needle, lysates were sonicated for 10 sec with a fine probe to shear genomic DNA. Then, 3×10^5 cell equivalents of each RNA was applied to a nitrocellulose filter (12 × 8 cm) using a 96-well blotting manifold and a 12-channel pipetter. Twenty replicate nitrocellulose filters were prepared from each set of 94 hybridoma RNAs. BW5147 RNA was applied to each filter and served as a positive control for constant region (C_β) expression and a negative control for all V_α and V_β probes. DNA probes [$V_\alpha 2$ (21), $V_\alpha 3$ (3), $V_\alpha 4$ (22), $V_\alpha 5$ (23), $V_\alpha 6$ (23), $V_\alpha 7$ (22), $V_\alpha 8$ (22), $V_\alpha 10$ (24), $V_\alpha 11$ (25), $V_\alpha 13$ (22), $V_\beta 2$ (23), $V_\beta 3$ (26), $V_\beta 4$ (23), $V_\beta 5$ (23), $V_\beta 6$ (27), $V_\beta 7$ (23), $V_\beta 8$ (23), $V_\beta 9$ (28), $V_\beta 10$ (23), $V_\beta 11$ (28), $V_\beta 12$ (28), $V_\beta 13$ (oligonucleotide), $V_\beta 14$ (29), $V_\beta 15$ (23), $V_\beta 16$ (23), $V_\beta 17$ (17), C_β (30), C_γ (31), C_δ (R. O'Brien and W. Born, personal communication) and CD4 (32)] were labeled by nick-translation (33) or random priming (34) and hybridized to hybridoma RNA by standard conditions (35). Filters were washed nonstringently in 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) at room temperature and stringently in 0.1× SSC at 50°C–55°C (36). These conditions do not distinguish individual members within a V_α or V_β gene family (data not shown). Some of these probes were generously provided by D. Loh ($V_\beta 9$, -11, -12), L. Hood ($V_\beta 14$, $V_\alpha 2$), D. Raulet ($V_\beta 6$, $V_\alpha 3$), S. Hedrick ($V_\alpha 10$, -11), J. Kappler and P. Marrack ($V_\beta 17$), D. Littman (CD4), W. Born (C_γ, C_δ), and R. O'Brien (C_δ).

Statistical Analysis of the Data. The entire distribution of V gene expression in the three panels of T-cell hybridomas was subjected to χ^2 analysis (36) and the V genes ($V_\alpha 3$, $V_\beta 5$, $V_\beta 11$, and $V_\beta 12$) making a major contribution to the total χ^2 value were identified. The data for the $V_\alpha 3$, $V_\beta 5$, $V_\beta 11$ genes were

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; V, variable; D, diversity; J, joining; C, constant.

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separately subjected to χ^2 analysis, while the data for the expression of the $V_\beta 12$ gene was subjected to a Fisher 2 × 2 test (36). From the χ^2 and Fisher values, probability (P) values were determined. A χ^2 analysis was also carried out on the data for C_δ expression in the three panels of hybrids.

RESULTS

Three Panels of T-Cell Hybridomas from MHC Congenic Mice. B10, B10.BR, and B10.Q Con A blasts were used to generate 555, 583, and 491 T-cell hybridomas, respectively. RNA was extracted from these hybridomas and analyzed for their expression of various V_α and V_β gene segments by standard hybridization techniques. The expression of $V_\alpha 1$ and $V_\beta 1$ were not analyzed since these genes are expressed in the fusion parent, BW5147, and should be present in every hybridoma. The overall characteristics of α- and β-chain RNA expression in this collection of random T-cell hybridomas is shown in Table 1. A small number of hybrids (1.4–4.9%) in each panel were excluded from the analysis because insufficient RNA was obtained (data not shown). Any hybrid expressing more than two V_α genes or more than two V_β genes was assumed to be nonclonal and was also eliminated from our analysis. From 3.3% to 9.9% of the hybrids in each panel were excluded on this basis (data not shown). The total number of hybrids considered into the data base are 485 from B10 mice, 536 from B10.BR mice, and 451 from B10.Q mice. Of these, 1.5–3.9% express C_γ RNA, while 1.5–12% transcribe C_δ RNA. Interestingly, 8.4–20% of the hybrids in these panels express neither a V_α nor a V_β gene. Hybrids that have lost chromosomes carrying their rearranged α- and β-chain genes would fall into this category. Some of these hybrids may actually express $V_\alpha 1$ and/or $V_\beta 1$; however, we were unable to determine the frequency of expression of $V_\alpha 1$ and $V_\beta 1$ genes in all 3 hybridoma panels since the fusion parent BW5147 expresses these gene segments. Finally, any hybrids expressing a γ/δ receptor might not express V_α or V_β genes.

Considering hybridomas that express at least one V_α and/or one V_β gene in the B10 ($H\text{-}2^b$), B10.BR ($H\text{-}2^k$), and B10.Q ($H\text{-}2^q$) panels, between 23% and 34% do not express a V_α gene. On the other hand, only 10–12% of these hybrids fail to express a V_β segment. A larger fraction (46–53%) of the hybrids in these panels express a single V_α gene, while 19–24% express two V_α genes. Sixty-eight percent of the hybrids in all 3 panels express a single V_β gene and 20–22% express two V_β gene segments. From 46% to 69% of the hybrids in these 3 panels express CD4 mRNA.

V_α and V_β Gene Expression in B10 Hybridomas. We have separately analyzed the frequency of V_α (and V_β) gene expression in B10 hybrids that express a single V_α (or V_β) gene and in B10 hybrids expressing two V_α (or two V_β) genes (data not shown). The frequency of expression of the various V_α gene families is similar among hybrids expressing one or two V_α genes. This is somewhat surprising since one-half of the V_α transcripts in the hybrids expressing two V_α genes should be nonfunctional and unselected, while a singly expressed V_α gene is presumably functional and encodes an α-chain protein.

A similar analysis of V_β gene expression in this collection of B10-derived hybridomas (data not shown) demonstrates that the $V_\beta 17$ gene segment is expressed in 9.8% of hybrids that express two V_β genes but in only 0.7% of hybrids expressing a single V_β gene. This observation can be explained by the fact that the B10 allele of the $V_\beta 17$ gene, $V_\beta 17b$, carries a stop codon at amino acid position 88 (37). Since this gene cannot generate a functional β-chain, a $V_\beta 17$ transcript is unlikely to appear in a functional T cell by itself but could appear as the nonfunctional transcript in a hybrid expressing two V_β genes. The fact that the $V_\beta 17$ gene is genetically nonfunctional in B10 mice allows us to estimate the fraction of sterile transcripts among hybrids expressing a single V_β gene. In theory, no receptor-bearing T cells expressing a single V_β gene should transcribe the defective $V_\beta 17$ segment; the 0.7% of hybrids expressing $V_\beta 17$ and no other V_β gene most likely represent hybrids that have lost the chromosome that carried a functional β-chain gene rearrangement. We have estimated that the fraction of hybrids expressing a nonfunctional transcript is <12% (0.7% × 16 V_β families = 11.2%) in our panels of hybridomas transcribing a single V_β gene. Thus, by only considering hybridomas that express a single V_α (or V_β) gene, a large fraction of the transcripts considered in the analysis should be functional.

V_α Gene Expression in MHC Disparate Mice. To obtain information about the MHC-driven selection of T cells, we compared a random sample of the TCR repertoires of B10 ($H\text{-}2^b$), B10.BR ($H\text{-}2^k$), and B10.Q ($H\text{-}2^q$) mice by examining the expression of V_α and V_β genes in our three panels of T-cell hybridomas. Only data from those hybrids that express a single V_α (or a single V_β) gene was considered. By excluding hybrids expressing two V_α (or two V_β) genes, a large fraction of the transcripts considered in the analysis should be functional (see above). The data in Table 2 compare the frequency of expression of V_α gene families in T cells derived from B10, B10.BR, and B10.Q mice. Several points can be made about these data. The frequency of expression of each V_α family is not equivalent and the relative expression of each V_α family is not necessarily related to the number of genes in each family. For example, the $V_\alpha 5$, $V_\alpha 6$, and $V_\alpha 13$ families each contain two genes (21, 22); however, the $V_\alpha 5$ and $V_\alpha 13$ families are expressed 4 times more often than the $V_\alpha 6$ family. This observation is apparent in all three hybridoma panels. Even more striking is the virtual absence of hybridomas expressing gene segments in the $V_\alpha 7$ family. Among 1900 hybrids we have examined (Table 2; unpublished observations), only 6 (0.3%) were found that express $V_\alpha 7$ genes.

Table 1. Characteristics of T-cell hybridomas from three strains of MHC congenic mice

Strain	B10 ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	B10.Q ($H\text{-}2^q$)
Hybrids	485	536	451
C_γ^+	19 (3.9%)	8 (1.5%)	13 (2.9%)
C_δ^+	59 (12%)	8 (1.5%)	40 (8.9%)
V_α^- and V_β^-	83 (17%)	45 (8.4%)	91 (20%)
V_α^+ and/or V_β^+	402	491	360
0 V_α	120 (30%)	113 (23%)	124 (34%)
1 V_α	186 (46%)	258 (53%)	167 (46%)
2 V_α	96 (24%)	120 (24%)	69 (19%)
0 V_β	49 (12%)	48 (10%)	42 (11%)
1 V_β	272 (68%)	334 (68%)	243 (68%)
2 V_β	81 (20%)	109 (22%)	75 (21%)
CD4 RNA$^+$	276 (69%)	273 (56%)	165 (46%)

Table 2. Distribution (%) of V_α gene expression in three panels of random T-cell hybridomas

	B10 ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	B10.Q ($H\text{-}2^q$)
$V_\alpha 2$	13	9.7	17
$V_\alpha 3$	<u>30</u>	23	<u>13</u>
$V_\alpha 4$	15	17	15
$V_\alpha 5$	8.6	8.1	11
$V_\alpha 6$	1.6	2.7	2.4
$V_\alpha 7$	<u>0.5</u>	<u>0</u>	<u>0</u>
$V_\alpha 8$	18	26	25
$V_\alpha 10$	4.3	3.1	3.6
$V_\alpha 11$	1.1	3.1	2.4
$V_\alpha 13$	7.5	7.8	9.6

Data are derived from 186 B10 hybrids, 258 B10.BR hybrids, and 167 B10.Q hybrids expressing a single V_α gene. The biological significance of underscored values is discussed in the text.

Finally, genes in the $V_\alpha 3$ family are expressed twice as often in T cells derived from B10 ($H\text{-}2^b$) mice as they are in T cells derived from B10.Q ($H\text{-}2^q$) mice ($P < 0.0004$). Interestingly, no other V_α gene family is differentially expressed in T cells from the MHC congenic strains we have examined.

V_β Gene Expression in MHC Disparate Mice. Unlike the V_α genes, most of the V_β segments are unique genes. The exceptions are the $V_\beta 5$ and $V_\beta 8$ families, which each contain three members (23, 38). The data in Table 3 compare B10 ($H\text{-}2^b$), B10.BR ($H\text{-}2^k$), and B10.Q ($H\text{-}2^q$) T cells for their expression of V_β genes. Again, the frequency of expression of a particular V_β gene segment is not related to the number of genes in a V_β family. For example, $V_\beta 6$ and $V_\beta 13$ are both single copy genes, yet in all three strains $V_\beta 6$ is expressed 10 times more frequently than is $V_\beta 13$. The frequent expression of $V_\beta 6$ has been noted (39). There are some striking differences in the expression of a few V_β gene segments among MHC disparate strains. Considering the total number of V_β genes expressed in each panel, the $V_\beta 5$ family accounts for 12% of the expressed V_β genes in B10 T cells, 9.1% of the expressed V_β genes in B10.Q T cells, but only 0.6% of the expressed V_β genes in B10.BR T cells [B10 vs. B10.BR ($P < 0.000008$); B10.Q vs. B10.BR ($P < 0.000008$)]. Two other V_β genes, $V_\beta 11$ and $V_\beta 12$, are also less frequently expressed in B10.BR ($H\text{-}2^k$)-derived T cells than they are in B10 ($H\text{-}2^b$)- or B10.Q ($H\text{-}2^q$)-derived T cells. Ten percent of B10.Q, 7% of B10, but only 2.4% of B10.BR T-cell hybrids express $V_\beta 11$. These differences are statistically significant [B10.Q vs. B10.BR ($P < 0.0004$) and B10 vs. B10.BR ($P < 0.05$)]. $V_\beta 12$ accounts for 4.6% of the B10.Q hybrids, 2.2% of the B10 hybrids, but none (0%) of the B10.BR ($H\text{-}2^k$) hybrids; these differences are statistically significant as well [B10.Q vs. B10.BR ($P < 0.000004$) and B10 vs. B10.BR ($P < 0.008$)].

γ and δ Expression. We also examined the expression of C_γ and C_δ genes in these hybridoma panels. The data in Table 4 show that 1.3–2.3% of all the hybrids (including those hybrids that express neither a V_α nor a V_β gene) express C_γ but not C_δ RNA. On the other hand, 10.5% of B10 ($H\text{-}2^b$), 8.2% of B10.Q ($H\text{-}2^q$), but only 1.3% of B10.BR ($H\text{-}2^k$) hybridomas (including hybrids that express neither a V_α nor a V_β gene) express C_δ but not C_γ RNA [B10 vs. B10.BR ($P < 0.000001$); B10.Q vs. B10.BR ($P < 0.000001$)]. The fraction of T cell hybrids that coexpress C_γ and C_δ is 1.6% in the B10 panel, 0.6% in the B10.Q panel, but only 0.19% in the B10.BR panel. This difference may be a reflection of the low frequency of C_δ expression in the B10.BR ($H\text{-}2^k$) hybridoma panel. The fraction of hybridomas coexpressing C_γ and C_δ RNA (0.19–1.6%) is somewhat lower than previous estimates (3%) of the fraction of peripheral T cells expressing γ/δ receptors (11).

Table 3. Distribution (%) of V_β gene expression in three panels of random T-cell hybridomas

	B10 ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	B10.Q ($H\text{-}2^q$)
$V_\beta 2$	6.6	12	6.2
$V_\beta 3$	3.7	4.8	2.1
$V_\beta 4$	5.9	5.7	8.2
$V_\beta 5$	12	<u>0.6</u>	9.1
$V_\beta 6$	13	13	9.5
$V_\beta 7$	3.7	5.7	2.9
$V_\beta 8$	25	34	26
$V_\beta 9$	2.6	2.4	2.5
$V_\beta 10$	5.5	4.5	3.7
$V_\beta 11$	7	<u>2.4</u>	10
$V_\beta 12$	2.2	<u>0</u>	5.8
$V_\beta 13$	1.8	2.4	0.8
$V_\beta 14$	2.6	4.5	1.6
$V_\beta 15$	2.6	3.3	3.3
$V_\beta 16$	5.1	3.3	7.8
$V_\beta 17$	0.7	0.9	0.4

Data are derived from 272 B10 hybrids, 334 B10.BR hybrids, and 243 B10.Q hybrids expressing a single V_β gene. The biological significance of underscored values is discussed in the text.

Table 4. C_γ and C_δ expression (%) in three panels of peripheral T-cell hybridomas

	B10 ($H\text{-}2^b$)	B10.BR ($H\text{-}2^k$)	B10.Q ($H\text{-}2^q$)
C_γ^+/C_δ^-	2.3	1.3	2.2
C_γ^-/C_δ^+	10.5	<u>1.3</u>	8.2
C_γ^+/C_δ^+	1.6	0.19	0.6

Data are derived from 485 B10 hybrids, 536 B10.BR hybrids, and 451 B10.Q hybrids. The biological significance of the underscored value is discussed in the text.

DISCUSSION

The early thymic TCR repertoire is a product of the rearrangement and expression of α- and β-chain gene segments to form functional α- and β-chains. Pairs of α- and β-chains are clonally distributed on thymocytes and this population of TCRs constitutes the "preselected" repertoire. Comparing MHC congenic strains, the preselected repertoire should be similar, since the α- and β-chain loci in these mice have the same genetic origin (B10) (40). On the other hand, the mature peripheral TCR repertoire is a product of positive and negative selection pressures acting on the preselected repertoire; the selection of T cells occurs in the thymus and is a result of the interaction of thymocytes (via their receptors) with MHC (15, 16, 18) and non-MHC (41, 42) gene products. Any differences in the peripheral T-cell repertoire between MHC congenic mice most likely reflect the influence of MHC-encoded proteins on the positive and negative selection of T cells. To characterize a portion of the selected T-cell repertoire in these three strains of MHC disparate mice, we have generated large panels of T-cell hybridomas from B10 ($H\text{-}2^b$), B10.BR ($H\text{-}2^k$), and B10.Q ($H\text{-}2^q$) mice and have analyzed the relative frequency with which they express particular TCR V_α and V_β gene segments.

Our experiments reveal the frequency of expression of V_α and V_β genes among random peripheral T cells in B10 ($H\text{-}2^b$), B10.BR ($H\text{-}2^k$), and B10.Q ($H\text{-}2^q$) mice, and establish a baseline of V gene expression in random peripheral T cells to which V gene expression in panels of antigen-specific or allospecific T cells can be compared. Such comparisons should prove useful in determining whether a particular V gene segment is over- or underutilized in a particular antigen response. Since these hybridoma panels provide a source of cell lines whose TCR V gene segments have been identified, these hybrids should also be useful in generating anti-TCR antibodies and characterizing their specificities.

Among hybridomas that express at least one TCR V gene (V_α and/or V_β), 23–34% do not express a V_α gene. On the other hand, only 10% of these hybrids fail to express a V_β segment (see Table 1). This may indicate that our collection of V_β probes is more complete than our collection of V_α probes (i.e., there may be some V_α genes that have yet to be identified). Alternatively, a large fraction of T cells may express genes in the $V_\alpha 1$ family, which we were unable to analyze given the presence of $V_\alpha 1$ RNA in BW5147. A larger fraction (46–53%) of the hybrids in our panels express a single V_α gene, while 19–24% express two V_α genes (see Table 1). This is similar to V_κ expression in normal B cells in that one-third of splenic B lymphocytes carry rearrangements of both J_κ loci and presumably express two V_κ genes (43).

This analysis identifies one V_α gene family ($V_\alpha 3$) whose expression varies among MHC disparate mice. Most V_α gene families, however, show no difference in their frequency of expression among MHC congenic strains (see Table 2). This may be due to the fact that most V_α gene families contain

several genes and the hybridization conditions used in these experiments do not discriminate between individual family members. Our analysis tends to average the expression of all V_α genes within a family and might not detect the variation of expression of an individual V_α gene between MHC congenic mice. The exception is the $V_\alpha 3$ gene family, which is expressed twice as frequently among B10 ($H\text{-}2^b$) T cells than it is among B10.Q ($H\text{-}2^q$) T cells ($P < 0.0004$). The $V_\alpha 3$ family contains approximately four genes (21) and it will be of interest to determine whether all $V_\alpha 3$ genes are more frequently expressed in B10 relative to B10.Q T cells or if this frequent expression in B10 T cells is confined to one or a few members of the $V_\alpha 3$ gene family. Another striking feature of the V_α analysis is the extremely infrequent occurrence of T cells expressing $V_\alpha 7$ genes. Only 6 of 1900 (0.3%) hybrids examined (Table 2; data not shown) expressed $V_\alpha 7$ genes. Given the fact that the $V_\alpha 7$ family comprises 10% of the V_α germ-line pool, there are 30 times fewer peripheral T cells expressing $V_\alpha 7$-encoded antigen receptors than would be expected. Since the D_δ–J_δ–C_δ complex is located on chromosome 14 between the V_α and J_α genes (14), V_α genes can potentially form functional δ-chain genes. In this light, it is conceivable that $V_\alpha 7$ genes preferentially, although not exclusively (21, 22), rearrange to the D_δ–J_δ–C_δ complex. Preliminary data with thymic hybridomas from newborn B10 mice (M. P. Happ and E.P., unpublished observations) support this idea.

Examination of V_β expression in these hybridoma panels identifies four V_β gene segments ($V_\beta 5.1$, $V_\beta 5.2$, $V_\beta 11$, and $V_\beta 12$) that are differentially expressed in T cells from MHC disparate mice (see Table 3). $V_\beta 5.1$ and $V_\beta 5.2$ are considered together together since these genes are homologous and the hybridization conditions used in these experiments do not distinguish them. $V_\beta 5.3$, a related pseudogene (38), is not detected under these conditions (data not shown). Considering the total number of singly expressed V_β genes in the three hybridoma panels, $V_\beta 5.1$ and $V_\beta 5.2$ comprise 12%, 9.1%, and 0.6% of the expressed V_β genes in B10 ($H\text{-}2^b$), B10.Q ($H\text{-}2^q$), and B10.BR ($H\text{-}2^k$) mice, respectively. These differences are statistically significant [B10 vs. B10.BR ($P < 0.000008$); B10.Q vs. B10.BR ($P < 0.000008$)]. Thus, the relative frequency of expression of these two genes is reduced by a factor of ≈20 in T cells from B10.BR ($H\text{-}2^k$) mice. This difference may be a function of $H\text{-}2^k$ gene products imparting a negative selection on these cells or of $H\text{-}2^b$ (or $H\text{-}2^q$) gene products imparting a positive selection on $V_\beta 5$-expressing T cells. Determination of the frequency of $V_\beta 5$-expressing T cells in (B10 × B10.BR)F_1 mice should clarify this point. If the frequency of $V_\beta 5$-expressing T cells is low in (B10 × B10.BR)F_1 mice [i.e., the B10.BR ($H\text{-}2^k$) phenotype is dominant], then the clonal deletion of $V_\beta 5$-expressing T cells by $H\text{-}2^k$ gene products is implicated. On the other hand, if the frequency of $V_\beta 5$-expressing T cells is high in (B10 × B10.BR)F_1 mice [i.e., the B10 ($H\text{-}2^b$) phenotype is dominant], then the positive selection of $V_\beta 5$-expressing T cells by $H\text{-}2^b$ gene products is a likely explanation. Two other V_β genes, $V_\beta 11$ and $V_\beta 12$, behave similarly to $V_\beta 5.1$ and $V_\beta 5.2$ (see Table 3). $V_\beta 11$ comprises 7%, 10%, and 2.4% of the expressed V_β genes in B10, B10.Q, and B10.BR mice, respectively. The lower frequency of $V_\beta 11$-expressing T cells in B10.BR mice is statistically significant [B10 vs. B10.BR ($P < 0.05$); B10.Q vs. B10.BR ($P < 0.0004$)]. $V_\beta 12$ comprises 5.8%, 2.2%, and none (0%) of the expressed V_β genes in hybridomas from B10.Q, B10, and B10.BR mice, respectively. These differences are statistically significant as well [B10 vs. B10.BR ($P < 0.05$); B10.Q vs. B10.BR ($P < 0.0001$)]. The mechanism regulating the frequency of $V_\beta 11$- and $V_\beta 12$-expressing T cells in these strains may be clarified by examining the frequency of these T cells in the relevant F_1 mice (see above).

It is intriguing that the frequency of $V_\beta 5.1$-, $V_\beta 5.2$-, $V_\beta 11$-, and $V_\beta 12$-expressing T cells is low in B10.BR mice relative to B10 and B10.Q mice and that B10.BR ($H\text{-}2^k$) mice express a functional I-E molecule while B10.Q ($H\text{-}2^q$) and B10 ($H\text{-}2^b$) mice do not (40). Recent experiments of Kappler *et al.* (17, 18) demonstrate reactivity for the class 2 MHC protein I-E among T cells bearing antigen receptors encoded by the $V_\beta 17a$ gene. In strains that express a functional I-E molecule, these T cells are clonally deleted. In this light, our experiments may indicate that several other V_β genes can impart I-E reactivity to their receptors and that these cells may be tolerized in I-E-bearing mice. Ongoing experiments demonstrate that T cells bearing $V_\beta 11$-encoded receptors are deleted from I-E-expressing mice (J.B., E.P., and O. Kanagawa, unpublished data). On the other hand, we have been unable to demonstrate significant I-E reactivity among 59 $V_\beta 5$-expressing T-cell hybridomas. These hybrids were tested for interleukin 2 production in the presence of B10 ($H\text{-}2^b$), B10.Q ($H\text{-}2^q$), and B10.BR ($H\text{-}2^k$) splenocytes and only two were found to be $H\text{-}2^k$ (and possibly I-E) reactive (J.B. and E.P., unpublished results). Thus, if $V_\beta 5$-encoded receptors are I-E reactive, then it is not apparent in T-cell hybridomas. Furthermore, if T cells expressing $V_\beta 5$-encoded receptors are found not to be generically I-E reactive, then another explanation for the absence of $V_\beta 5$-expressing T cells in B10.BR ($H\text{-}2^k$) mice is required.

Garman *et al.* have carried out a similar set of experiments (27) using RNase protection assays with three V_α and three V_β probes to compare the extent of particular V_α and V_β gene segment expression in T-cell RNA from BALB/c ($H\text{-}2^d$), BALB.B ($H\text{-}2^b$), and BALB.K ($H\text{-}2^k$) mice. Using probes for the $V_\alpha 1$, $V_\alpha 3$, and $V_\alpha 4$ gene families and the $V_\beta 6$, $V_\beta 7$, and $V_\beta 8$ genes, no significant differences were found between MHC disparate mice. Our own results agree with the findings of Garman *et al.* considering the probes that were used. Nevertheless, the present analysis reveals a 2-fold difference in the frequency of $V_\alpha 3$-expressing T cells between B10 and B10.Q mice. Garman *et al.* may not have identified this difference since their experiments compared BALB MHC congenics, and $H\text{-}2^q$ mice were not examined.

These data also reveal a difference in the frequency of T cells coexpressing C_γ and C_δ RNA between B10 ($H\text{-}2^b$), b10.Q ($H\text{-}2^q$), and B10.BR ($H\text{-}2^k$) mice: 1.6% of B10-derived hybrids, 0.6% of B10.Q-derived hybrids, but only 0.19% of B10.BR-derived hybrids coexpress C_γ and C_δ RNA. The frequency of γ/δ-expressing T-cell hybrids from B10 and B10.Q mice is consistent with, albeit somewhat lower than, estimates of the fraction (3%) of γ/δ-receptor-bearing T cells in the periphery (11). In addition, there is a striking difference between B10, B10.Q, and B10.BR hybrids that express C_δ but not C_γ RNA [i.e., there are 8 times fewer γ^-/δ^+ hybrids in the B10.BR ($H\text{-}2^k$) panel]. Thus, the low frequency of γ^+/δ^+ hybridomas generated from B10.BR mice is best explained by the low frequency of C_δ-expressing B10.BR hybrids in general (see Table 4). At face value, these data may suggest that $H\text{-}2^k$ gene products select against T cells expressing δ-chains even in the absence of γ-chains. We do not favor this interpretation since many of the δ-chain RNAs in these hybrids are likely to be nonfunctional. One must also consider the possibility that $H\text{-}2^k$ gene products select against T cells expressing δ-chain RNA, although the mechanism by which this might occur is unclear.

Finally, these experiments reveal significant differences in the relative contributions of one V_α gene family ($V_\alpha 3$) and several V_β gene segments ($V_\beta 5.1$, *-5.2*, *-11*, and *-12*) to the TCR repertoire of MHC disparate mice. These data support the idea that MHC gene products have an impact on the composition of the TCR repertoire and suggest that, in some cases, a single receptor element (V_α or V_β) determines the fate of a T cell.

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