III/LAV–infected cells were used instead of cell-free virus (*22*).

Sodroski *et al.* reported that gene expression directed by the LTR sequence of HTLV-III is stimulated only in cell lines infected with HTLV-III and not with HTLV-I or -II (*23*). Gallo and his colleagues showed that an HTLV-I–positive cell line, C91/PL, was susceptible to HTLV-III infection although H9 cells were more efficient in terms of viral replication (*9*). Using a large panel of HTLV-I–positive cell lines of human and monkey origin, we determined whether the efficient replication of HTLV-III observed in MT-2 and MT-4 cells is a general phenomenon in HTLV-I–positive cell lines. Thirteen of 21 cell lines showed more rapid appearance and increase of viral antigens after HTLV-III infection than the H9 cells used as a control. However, HTLV-III replication efficiency did not appear to be correlated with the frequency of the cells positive for the HTLV-I antigens detectable by IF (*24*). Thus it is not known whether HTLV-III replication after infection is correlated with LOR gene expression in these HTLV-I–carrying cells.

It is also not known whether the virus produced from HTLV-I–positive cell lines after HTLV-III infection are phenotypically altered [an interaction has been observed between HTLV-I and vesicular stomatitis virus (VSV) (*25*)]. Cell-free HTLV-I usually fails to infect and transform normal lymphocytes, although there are some exceptions ascribed to the labile envelope of the virus (*26*). We found no biological activity of HTLV-I in filtrates of the culture medium of MT-2 and MT-4 cells after HTLV-III infection; however, the biological activity of HTLV-III seemed to be enhanced (*27*).

The establishment of a system that permits rapid and efficient replication of HTLV-III and cell death opens the way to the routine detection and isolation of HTLV-III from the infected patients and may facilitate studies of the AIDS virus.

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T-Cell Receptor β-Chain Expression: Dependence on Relatively Few Variable Region Genes

Abstract. *Fifteen independently isolated complementary DNA clones that contain T-cell receptor (TCR) V_β genes were sequenced and found to represent 11 different V_β genes. When compared with known sequences, 14 different V_β genes could be defined from a total of 25 complementary DNA's; 11 clones therefore involved repeated usage of previously identified V_β's. Based on these data, we calculate a maximum likelihood estimate of the number of expressed germline V_β genes to be 18 with an upper 95 percent confidence bound of 30 genes. Southern blot analysis has shown that most of these genes belong to single element subfamilies which show very limited interstrain polymorphism. The TCR β-chain diversity appears to be generated from a limited V_β gene pool primarily by extensive variability at the variable-diversity-joining (V-D-J) junctional site, with no evidence for the involvement of somatic hypermutation.*

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The vertebrate immune system characteristically responds to a broad range of foreign antigens with great specificity. The mechanisms through which diversity and clonal specificity are generated have been described in great detail for the B-cell receptor, or antibody molecule. Antibody diversity is generated by (i) somatic recombination between a large pool of germline variable (V) elements and germline diversity (D) and joining (J) elements to produce a functionally rearranged immunoglobulin gene; (ii) variation in the precise sites of this joining event; (iii) insertion of random N region nucleotides at the junctional sites; and (iv) somatic hypermutation (*1*).

Recent findings have shown that the T-cell receptor (TCR) is a heterodimer (*2–4*), each chain of which is the product of a somatic DNA rearrangement similar to that described for immunoglobulin genes (*5–8*). The germline organization of the murine D_β, J_β, and C_β elements has been described (*5, 9–12*). Two closely linked constant region (C_β) elements exist, both of which are preceded by six functional J_β elements; two D_β elements have been localized thus far, with one D_β preceding each of the two J_β clusters. An unknown number of V_β genes are presumed to lie 5′ to the D_β, J_β, and C_β gene clusters.

In order to define the repertoire of V_β genes and assess their role in generating diversity in the T-cell receptor β chain, we examined a series of V_β containing complimentary DNA (cDNA) clones. Sequence analysis of these clones leads us to conclude that the number of functionally expressed germline V_β genes is relatively small, and that the generation of β chain diversity primarily involves variability introduced by the V-D-J rearrangement events.

To assess available diversity among mouse TCR V_β chains, we isolated and sequenced 15 independent V_β-containing clones from cDNA libraries screened with a TCR C_β-specific probe, including four from BALB/c thymus, nine from

C57BL/6 spleen, one from a T-helper hybridoma (T_H), and one from a T-killer cell line (T_K). Thirteen of these isolates appear to encode functional TCR β-chain polypeptides. Of the remaining two, V_β 10 has a stop codon at amino acid position 58, while the VDJ joining events of V_β7 frameshift the downstream J_β and C_β regions; thus both are unable to encode a full-length functional polypeptide.

Sequence comparison has shown that

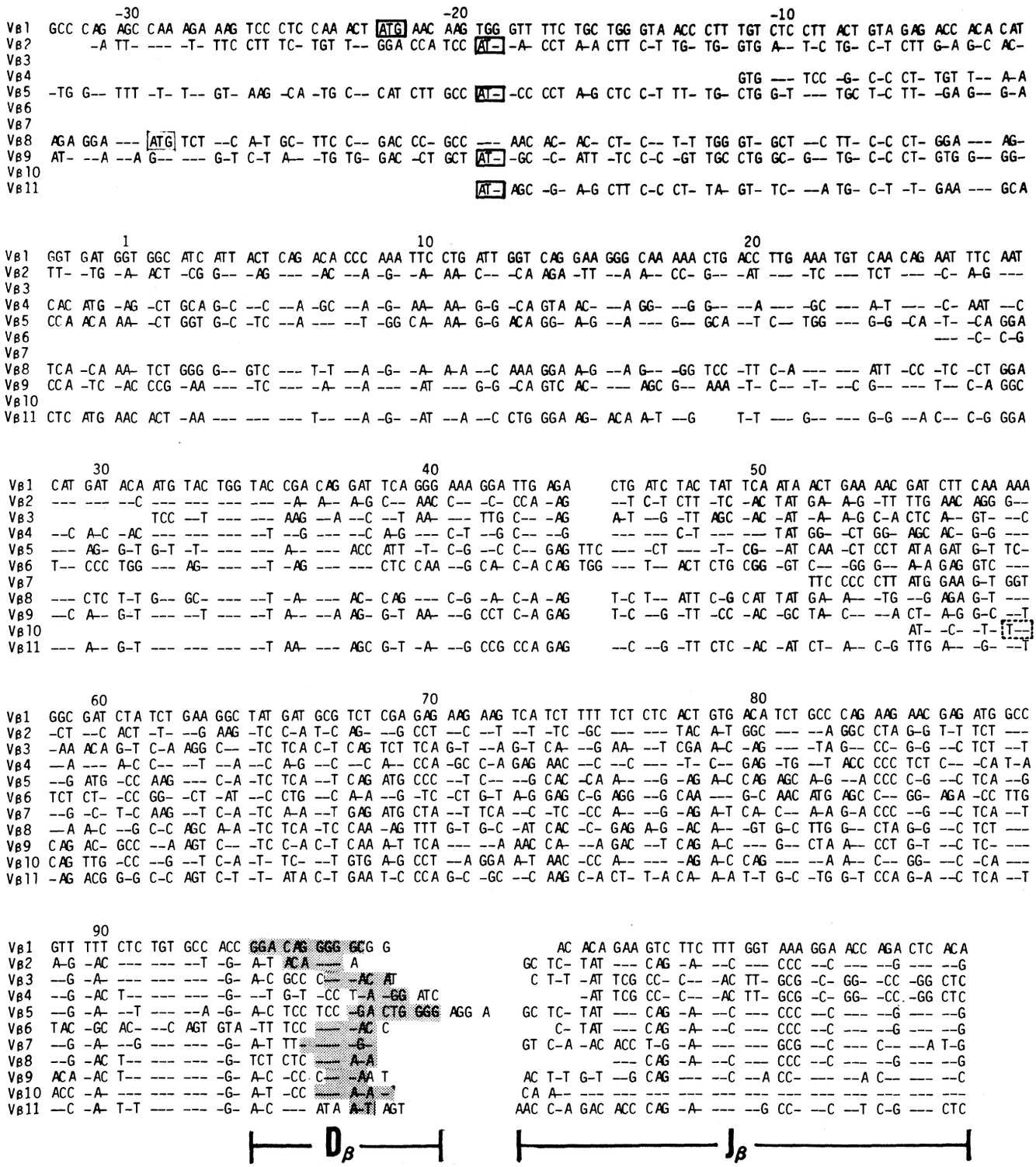

Fig. 1. DNA sequence of the VDJ regions from 11 cDNA isolates. Total cellular RNA was prepared from two cell lines: F3, T_K clone against C57BL/6 (*35*), DA33 C2, a B6 T_H hybridoma fused with BW5147 (*36*), and C57BL/6 spleen Con-A (Concanavolin A) blasts by the guanidium isothiocyanate method (*37*). Complementary DNA libraries were constructed in the λgt10 cloning vector (*38*), with minor modifications (*39*). These three libraries, together with a BALB/c thymus cDNA library in λgt10 (*40*) were screened with a murine C_β containing cDNA clone (*41*). C_β positive cDNA's were subcloned into pUC 12 and sequenced by the method of Maxam and Gilbert (*42*). A dash (−) indicates base identity with the top line. Codon numbering follows that of Patten *et al.* (*13*). Where possible, putative initiation codons are boxed (solid lines). The stop codon in V_β10 is also boxed (broken line). Sequences contributed from germline D_β1.1 or D_β2.1 elements are highlighted, and J_β gene segments are aligned separately. Bases between D_β and J_β segments are proposed N region insertions.

these 15 V_β isolates represent 11 distinct V_β gene segments with four duplicates. The sequences of our 11 different V_β genes are presented in Fig. 1 (duplicates are not included). The V_β–D_β and D_β–J_β junctional sequences are distinct in each of the duplicate pairs and therefore these must represent independent yet repeated usage of the same V_β gene segments (data not shown); thus, 27 percent (4 of 15) of our V_β isolates were independent duplicates. This high rate of redundancy becomes more striking when our data are combined with those reported by others, as summarized in Table 1. Twenty-five independent V_β cDNA sequences are now available representing 14 different V_β gene segments, resulting in a duplication rate of 44 percent.

The observed high incidence of redundancy in V_β gene usage implies that either (i) there exists a small pool of V_β genes in the mouse genome or (ii) a small subset of V_β genes from a larger pool is repeatedly used, as a result of either antigen selection or developmental programming. In either case, the number of functionally expressed V_β gene segments accounting for the majority of T-cell receptor V_β-chains must be relatively small. Patten and his colleagues noted earlier that a small number of V_β predominate in the thymus (*13*) while Goverman *et al.* suggest further that the germline pool of V_β gene segments may be small (*14*). There are enough V_β isolates available now to estimate the size of this gene pool. Since the 25 V_β genes in Table 1 were obtained from as diverse sources as are available (helper T cells, killer T cells, splenic T cells, and thymocytes from a variety of mouse strains), we believe that the database approximates a random sampling of V_β genes. While this might not be strictly the case, the sampling does represent all V_β's available to us. We therefore estimated the maximum probable gene pool size, assuming that there was no significant skewing of the database by nonrandom V_β utilization.

For a fixed number n of distinct V_β gene sements in the genome, each equally likely to be sampled, the probability of observing exactly d distinct gene segments among a total number r examined is (*15*)

$$P(d) = S(r,d)\binom{n}{d} d!\, n^{-r}$$

where the $S(r,d)$ are Stirling's numbers of the second kind (*16*). The maximum likelihood estimate of n is $n = 18$, which was obtained by determining the value of n that maximized $P(d)$ for d and r fixed at $d = 14$ and $r = 25$. A 95 percent one-sided confidence bound of $n = 30$ was obtained by determining the smallest value of n for which the probability of observing at most 14 different segments among 25 segments examined was less than 0.05.

The observed distribution is most consistent with an expressed V_β gene pool size of 18 with a 95 percent confidence bound of 30. While the actual number of germline V_β's will be larger than we have calculated if all V_β genes are not equally expressed, the data nevertheless indicate that the expressed V_β gene pool is relatively small when compared with the large immunoglobulin V_K or V_H families of 200 to 250 genes (*1*). Thus a small number of germline V_β gene segments (less than 30) account for the bulk of the T-cell receptor β-chain repertoire seen in the mouse.

This analysis is based on the assumption that individual strains of mice have similar if not identical genetic content at the V_β loci. Of the 11 V_β sequences presented in Fig. 1, eight are new isolates while three are virtually identical to previously published V_β sequences. Of these three, $V_\beta 1$, isolated from the BALB/c thymus cDNA library, differs from the LB2 gene (*13*) derived from a C57BL/6 helper T-cell line by only one nucleotide over a total of 480 bases compared.

The $V_\beta 11$, of either AKR or C57BL/6 origin, differs from the published BALB/c 86T1 sequence (*17*) at one base. Similarly, $V_\beta 6$ isolated from C57BL/6 spleen differs from the BALB/c E1 gene (*13*) by only two nucleotides over a total of 200 bases compared. In contrast to the previous three comparisons, our BALB/c $V_\beta 4$ gene shows only 91 percent homology to the previously identified C57BL/6 C5 gene (*13*). Because Southern blots that were hybridized with $V_\beta 4$ and C5 (*13*) reveal three and two bands respectively, it is possible that $V_\beta 4$ and C5 represent different members of a two- to three-member subfamily and are therefore pseudoalleles. We conclude that V_β allelic counterparts

Table 1. Summary of V_β isolates and their sources (*34*). Abbreviations B/c, BALB/c; B6, C57BL/6; Ty, thymocyte; and Sp, spleen.

V_β gene	Isolates	Source
1	4	LB2 (B6 T_H) (*13*), LB2-Like (T_K)[a], $V_\beta 1$ (B/c Ty)*, $V_\beta 1.2$ (B6 Sp)*
2	1	$V_\beta 2$ (B/c Ty)*
3	1	$V_\beta 3$ (B/c Ty)*
4	1	$V_\beta 4$ (B/c Ty)*
5	2	$V_\beta 5$ (B6 Sp)*, F3β (B6 T_K)*
6	3	E1 (B/c T_H) (*13*), $V_\beta 6$ (B6 Sp)*, $V_\beta 6.2$ (B6 Sp)*
7	1	$V_\beta 7$ (B6 Sp)*
8	1	$V_\beta 8$ (B6 Sp)*
9	2	$V_\beta 9$ (B6 Sp)*, $V_\beta 9.2$ (B6 Sp)*
10	1	$V_\beta 10$ (B6 Sp)*
11	3	86T1 (B/c Ty) (*17*), 86T1-Like[a], $V_\beta 11$ (B6 T_H)*
12	2	C5 (B6 T_H) (*13*), C5-like (B6 T_K)[b]
13	2	2B4 (B10 T_H) (*5*), 3H-25 (B6 T_H) (*14*)
14	1	pHDS11 (B/c T_K) (*43*)

[a]Hedrick, in Patten *et al.* (*13*). [b]Tak Mak, personal communication. *This report.

Table 2. Summary of genomic Southern blot analysis. Abbreviations: N, nonpolymorphic; P, polymorphic; –, no band visualized.

Probe	Bands visualized (No.)	Mouse	Character by fragment length			
			Eco RI	Msp I	Pvu II	Sac I
$V_\beta 1$	1	BALB/c, B6 C3H, PL	N	N	N	N
		SJL	N	P	N	N
$V_\beta 2$	1	BALB/c, B6 C3H, PL	N	N	N	N
		SJL	–	–	–	–
$V_\beta 3$	1	BALB/c, B6 C3H, PL	N	N	N	N
		SJL	P	P	P	P
$V_\beta 4$	3	BALB/c, B6 C3H, PL	N	N	N	N
		SJL	–	–	–	–
$V_\beta 5$	1	BALB/c, B6	N	N	N	N
		SJL	–	–	–	–

are highly homologous between mouse strains.

To further assess possible interstrain variability, we examined various strains of inbred mice for restriction fragment length polymorphisms using isolated V_β genes as probes. Strains were chosen to include mice known to be of diverse ancestry (*18*), with different H-2 haplotypes or Lyt2 alleles (or both); this was done because both the C_β and Lyt2 loci have been mapped to the same chromosome (*19, 20*), and therefore the Lyt2 allele is included as a convenient marker for variation at chromosome 6. Strains used were BALB/c (H-2^d, Lyt2.2), C57BL/6 (H-2^b, Lyt2.2), C3H (H-2^k, Lyt2.1), PL (H-2^u, Lyt2.1), and SJL (H-2^s, Lyt2.2). Subcloned V region sequences derived from $V_\beta 1$ through 5 were used to probe Southern blots (*21, 22*) of liver DNA from the five mouse strains digested with four restriction endonucleases, Eco RI, Msp I, Pvu II, and Sac I (Table 2).

The mouse strains BALB/c, C57BL/6, C3H, and PL all gave identical results with probes V_β 1, 2, 3, and 5, showing single bands on the Southern blots which were nonpolymorphic with all enzymes employed. As was discussed earlier, probe $V_\beta 4$ hybridized to three bands, all of which were nonpolymorphic in these four strains. The results with SJL were very different from the other four strains. The SJL DNA gave no detectable hybridization with probes $V_\beta 2$, $V_\beta 4$, or $V_\beta 5$; it showed one band with $V_\beta 1$ which was polymorphic with one of the enzymes tested; $V_\beta 3$ hybridized to one band in SJL, which was polymorphic with all enzymes tested.

In summary, Southern blot analysis with isolated V_β probes reveals that most of them hybridize to a single band (the exception being $V_\beta 4$ with three bands), implying that most V_β gene segments comprise a single element subfamily within the larger V_β gene family. Comparison of the blot patterns of four inbred strains of distinct origins reveals no restriction fragment length polymorphism. These data imply that the total number of available V_β genes is limited, and that these genes are highly conserved within the inbred mouse population (see later discussion of the SJL mouse). It would be interesting to see if the V_β gene pool in man is as nonpolymorphic and limited in size as has been found in the mouse. It has been reported that the human C_β locus is highly conserved, with only a single individual showing a restriction fragment polymorphism out of 24 examined (*20*).

The T-cell antigen receptor is a heterodimer with specific reactivity toward antigen in association with self-MHC (*23, 24*). In that the contribution of germline V_β gene segments to this specificity is limited, most likely to less than 30 genes, any significant contribution to T-cell receptor repertoire diversity by the β chain must involve a large element of junctional variability. Rearrangements involving the 12 functional germline J_β sequences and two known D_β sequences together with random N region insertions can generate a large repertoire of functionally rearranged genes in the presence of a limited number of germline V_β gene segments. In addition, the cDNA clones presented here show a large degree of variation in precise recombination events involving D_β elements, with as many as 11 bases contributed by $D_\beta 1.1$ to the rearranged $V_\beta 1$ gene and as few as four bases contributing to the rearranged $V_\beta 9$ gene (Fig. 1).

Unlike immunoglobulin genes, T-cell receptor β genes give no evidence for extensive somatic hypermutation. In instances where the same V_β gene segments are used in independent clones from the same strain of mouse, the V_β-derived segments are identical; isolated single-base differences found in sequenced clones to date are most likely due to minor polymorphisms of germline genes among different inbred strains of mice. Thus, although low-level somatic mutational events cannot be ruled out, their extensive contribution to V_β sequence diversity seems unlikely.

To quantitatively assess regions of variability, we have performed a Wu-Kabat variability analysis (*25*) of 11 full-length V_β cDNA clones (Fig. 2a). In addition to areas of increased variability encoded in the germline V_β segments, significant variability is introduced by junctional events involving the D_β and J_β elements. For comparison, Fig. 2b shows a similar analysis performed on the 12 functional germline $J_\beta 1$ and $J_\beta 2$ segments. It is of interest that even in the germline, significant variability exists and is focused at the 5′ junctional end of the J regions. When this analysis is repeated including duplicate isolates of the available V_β's, variability at the V-D-J junctional region increases relative to the germline encoded variability, reflecting the capacity of somatic gene rearrangements to expand upon germline encoded variability (*26*). On the basis of these observations and the extensive potential of junctional, combinatorial, and N-region insertional elements to introduce clone-specific receptor variation, we conclude that the capacity to generate diversity in this region is more extensive than in the V_β-encoded region.

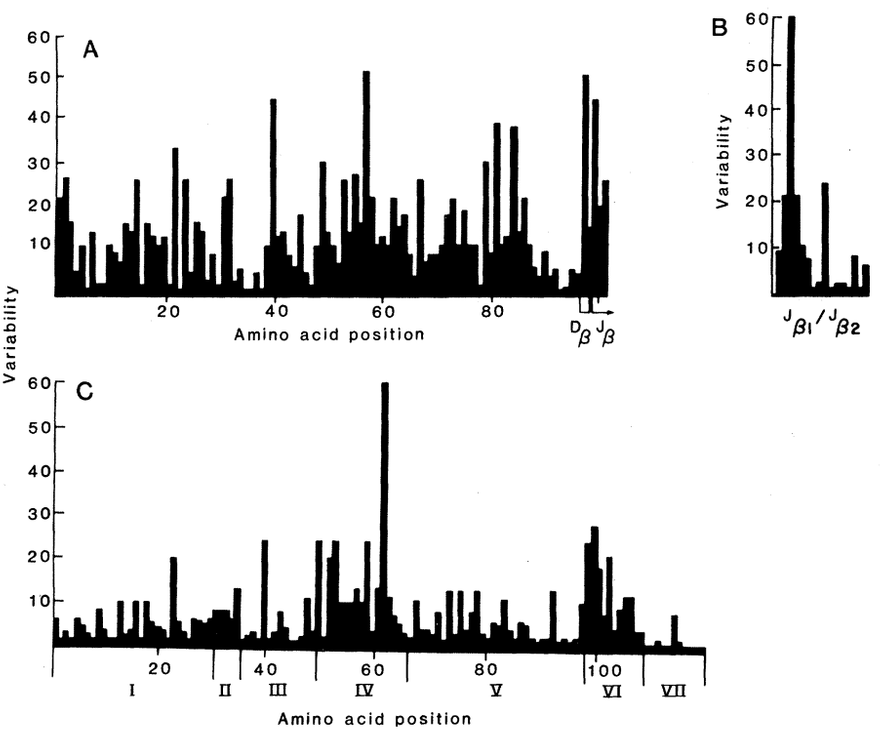

Fig. 2. Wu-Kabat variability plots. (A) The translated sequence of 11 full-length V_β genes including $V_\beta 1$, $V_\beta 2$, $V_\beta 4$, $V_\beta 5$, $V_\beta 8$, $V_\beta 9$, $V_\beta 11$, E1, 2B4, C5, and pHDS11. (B) The translated sequence of the 12 functional J_β segments. (C) The translated sequence of eight V_H genes from separate subfamilies, including B1-8 (μ), MOPC 21 (γ_1), 17.2.25 (γ_1), HPCM1 (μ), A/J ars (γ_1), MOPC 315 (α), MOPC 141 (γ_{2b}), and J606 (γ_3). Classical framework regions 1, 2, 3, and 4 are located at positions I, III, V, and VII while hypervariable regions 1, 2, and 3 are located at positions II, IV, and VI.

The variability analysis can be extended by comparing the plot of V_β variability (Fig. 2a) with that of immunoglobulin V_H variability (Fig. 2c), which was generated from eight V_H genes chosen from separate subfamilies (*25*). As noted by Patten *et al.* (*13*), the most striking feature in this comparison is the lack of extended framework regions in the TCR β-chain sequences. In contrast to immunoglobulin, TCR V_β chain contains isolated residues that are highly conserved amidst areas of low-level variability, rather than regions of extended framework.

Analysis of three pairs of V_β sequences suggests that the allelic counterparts of V_β genes among different inbred strains of mice are extremely similar to each other. Combining this fact with the absence of restriction fragment length polymorphism seen on limited Southern blots, we conclude that most inbred mouse strains share virtually identical V_β genomic content. The lack of divergence between strains can be explained in two possible ways: (i) the mouse strains used here were derived from a common ancestor at the time of inbreeding or (ii) selection against change is occurring in each V_β gene. For comparison, as an example of another small gene family, Vλ1 of BALB/c and C57BL/6 mice have been found to be identical (*27*). Furthermore, we have noted that the genomic J_H, D_β, and J_β and their flanking sequences differ between the two mouse strains by less than 1 percent (*28*), while others have determined that the immunoglobulin $C_{\gamma 2b}$ alleles differ between C57BL/6 and BALB/c by 0.4 percent (*29*). Using a value of 1 percent change per 0.7 million years (*30*), we can estimate an evolutionary separation of perhaps 300,000 years between strains at the $C_{\gamma 2b}$ locus. In this context, the observed < 1 percent divergence rate between allelic V_β genes indicates neither rapid divergence nor extensive conservation in the V_β gene system (*30*).

Extending this analysis to V_β genes from different species, one of our 11 different murine V_β sequences appears to be very similar to a published human V_β gene. We find our mouse $V_\beta 5$ gene and the published human YT35 (*31*) to be 68 and 78 percent homologous at the amino acid and nucleotide levels, respectively; the changes at the nucleotide level represent a replacement to silent ratio of 1.3 at the amino acid level. The fact that $V_\beta 5$ and YT35 are possible homologs between man and mouse suggests that both may have been derived from a common ancestral gene present at the point of divergence of man and mouse. If this is the case, the observed degree of divergence suggests an evolutionary history in which the primordial gene accumulated isolated simple mutations with moderate selection against replacement changes.

It has been suggested by others that some of the V_β single gene subfamilies (LB2, 2B4, 86T1, and E1) have undergone rapid interspecies sequence divergence, as assessed by Southern blot analysis, while others (C5 family) appear to cross-hybridize between man and mouse (*13*). Our $V_\beta 5$ gene appears to be an example of this latter category which has maintained significant homology between distant species. In the absence of selective pressure to maintain large blocks of conserved framework regions in V_β genes, it is not surprising that most V_β homologs fail to cross-hybridize between species. In fact, this lack of conserved framework prevents the cross-hybridization of nonallelic V_β genes within the same species. Our sequence comparison data are most consistent with V_β evolution occurring through simple mutational drift with little pressure to maintain framework.

The SJL mouse is an interesting anomaly. Of the five V_β probes used in Table 2, seven bands are found in the common mouse strains while only two bands are seen in SJL. We can interpret this result in two ways. First, it may indicate that a massive deletion event covering ~70 percent of the V_β locus has occurred in the SJL mouse. Such a scenario would predict that SJL may show "holes" in its T-cell repertoire when compared to BALB/c or C57BL/6 mice and therefore may be an immunologically compromised animal. Alternatively, SJL may have a complete set of V_β genes, many of whose members are distinct from those present in BALB/c, C57BL/6, C3H, or PL mice.

Interestingly, this is not the first demonstration that the TCR β chain of SJL mice is different from other common strains. Roehm *et al.* prepared a monoclonal antibody (KJ16-133) against an allotype and showed that it reacts with the receptor on 20 percent of T cells in most mice, but that it does not react with any T cells from SJL mice (*32*). This trait has now been mapped to the β-chain locus (*33*). We suggest here that the KJ16-133 reagent may be reacting to a determinant present on a subset of V_β genes which together represent 20 percent of the expressed V_β's in BALB/c or C57BL/6 mice, and that these genes are among those missing from the SJL genome.

In addition to its not recognizing the T cells of the SJL mouse, the KJ16-133 antibody does not react with T cells from C57BR, C57L, or SWR mice (*33*). Genomic Southern blot analysis of these strains indicates that they share the V_β deletion event described here for the SJL mouse (*28*), further supporting the conclusion that KJ16-133 is directed against a V_β allotypic marker.

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