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Diversity and structure of human T-cell receptor β-chain variable region genes

(nucleotide sequence/repertoire size/gene subfamilies/somatic mutation)

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ABSTRACT **The nucleotide sequences of 27 T-cell receptor β cDNA clones isolated from a human peripheral lymphocyte library were determined and compared to five additional published sequences. These cDNA clones represent 22 distinct T-cell receptor β-chain variable region (V_β) gene segment sequences, which fall into 15 different V_β gene subfamilies, each containing six or fewer members. From this analysis, we estimate that the repertoire of expressed human V_β genes is <59, apparently much smaller than the immunoglobulin heavy chain and light chain variable region (V_H and V_L) repertoires. Variability plots comparing these human V_β regions with each other and with published mouse V_β regions provide evidence for only four hypervariable regions homologous to those seen in comparisons of immunoglobulin V regions. Somatic hypermutation appears to be used infrequently, if at all, in these V_β genes.**

T-cell receptors in mammals are capable of responding to a wide range of foreign antigen determinants and, like their immunoglobulin counterparts, are enormously diverse. They are cell-surface heterodimers composed of α and β chains that are divided into variable (V) regions that bind antigen and major histocompatibility complex (MHC) determinant and constant (C) regions that are attached to the membrane (1, 2). Extensive studies in the mouse and in humans suggest that the V_β region is encoded by three distinct gene segments, *V*, diversity (*D*), and joining (*J*), which rearrange and join to generate a complete V_β gene in the differentiated T cell (3–7). Mouse β-chain genes have at their disposal all of the diversification mechanisms that are used by immunoglobulin genes but one. These include germ-line diversity, the maintenance of a multiplicity of *V*, *D*, and *J* gene segments, combinational diversity, the joining of available gene segments in all possible combinations, and somatic diversification (8, 9). Somatic diversification includes junctional diversity, the flexible joining of gene segments at different sites (10, 11), and N-region diversification, the addition of random nucleotides at rearranged gene segment junctions (10, 12). Somatic hypermutation, an increased frequency of nucleotide substitution occurring in and around immunoglobulin *V* genes (13, 14), occurs very infrequently, if at all, in T-cell receptor genes (15–18).

Two features distinguish the diversity patterns in mouse V_β genes from those of their immunoglobulin counterparts. First, there appear to be only 25–30 highly expressed mouse V_β gene segments (15–17) contrasting with the several hundred to 1000 *V* gene segments in the V region light chain and heavy chain (V_L and V_H) immunoglobulin gene families (19-21). Second, the published mouse V_β gene segments fall into 16 V_β subfamilies, as defined by nucleic acid hybridization with germ-line DNA on Southern blots, of which 13 contain just a single member (15–17, 22). In contrast, the immunoglobulin V_H and V_κ subfamilies, generally defined as sets of *V* gene segments ≥75% homologous (23), range in size from 4 to 1000 (9–21).

Conflicting analyses have been presented concerning the question of the number of hypervariable regions in mouse β chains. Hypervariable regions can be defined as sets of residue positions whose average variability is substantially greater than the mean variability of the entire sequence. Immunoglobulin V_L and V_H regions each have three "classically" defined hypervariable regions, which fold to constitute the walls of the antigen-binding site (24). Capra and Kehoe (25) have demonstrated that human V_H genes contain a fourth hypervariable region. Patten *et al.* (17) have argued that the V_β regions have substantially more than these four hypervariable regions (17), perhaps in keeping with the dual requirement of the T-cell receptor to recognize antigen in the context of a MHC molecule. In any case, much of the diversity in the β chain seems to be focused at the carboxyl end of the V region through the heavy use of junctional and N-region diversity, the unique ability to use D_β segments in all three translational reading frames, and the use of a large family of J_β segments that are, on average, considerably more diverse than J_H or J_κ segments (15–17).

We have analyzed the expression of β-chain genes in human peripheral lymphocytes in order to ascertain whether the strategies for diversification observed in mouse β chains are a standard feature of the T-cell immune response in higher vertebrates. We conclude that (*i*) the strategy of maintaining a limited repertoire of highly heterogeneous V_β gene segments is shared between mice and humans; (*ii*) there is no evidence for unique hypervariable regions in V_β genes; (*iii*) somatic hypermutation of human V_β genes occurs at a very low frequency, if at all; and (*iv*) somatic diversification of the carboxyl end of V_β regions play an important role in the somatic generation of V_β-region diversity.

MATERIALS AND METHODS

cDNA Library Construction. Human peripheral lymphocytes obtained from normal donors were purified on Ficoll gradients and stimulated for 72 hr with phytohemagglutinin. Poly(A)$^+$ RNA was isolated by the guanidinium thiocyanate method (26) followed by one round of selection on oligo(dT) cellulose. Oligo(dT)-primed cDNA was synthesized by the method of Huynh *et al.* (27). Double-stranded cDNA bearing *Eco*RI linkers was fractionated on a 1.2% agarose gel. Fractions ranging from 1 to 4.5 kilobases (kb) were ligated into *Eco*RI-cut λgt10. The resulting library contained 3.8 × 10^7 independent recombinant clones.

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Abbreviations: kb, kilobase(s); V_H, immunoglobulin heavy-chain variable region; V_L, immunoglobulin light-chain variable region; V_β, D_β, J_β, and C_β, variable, diversity, joining, and constant regions of the β chain of the T-cell receptor, respectively; MHC, major histocompatibility complex.

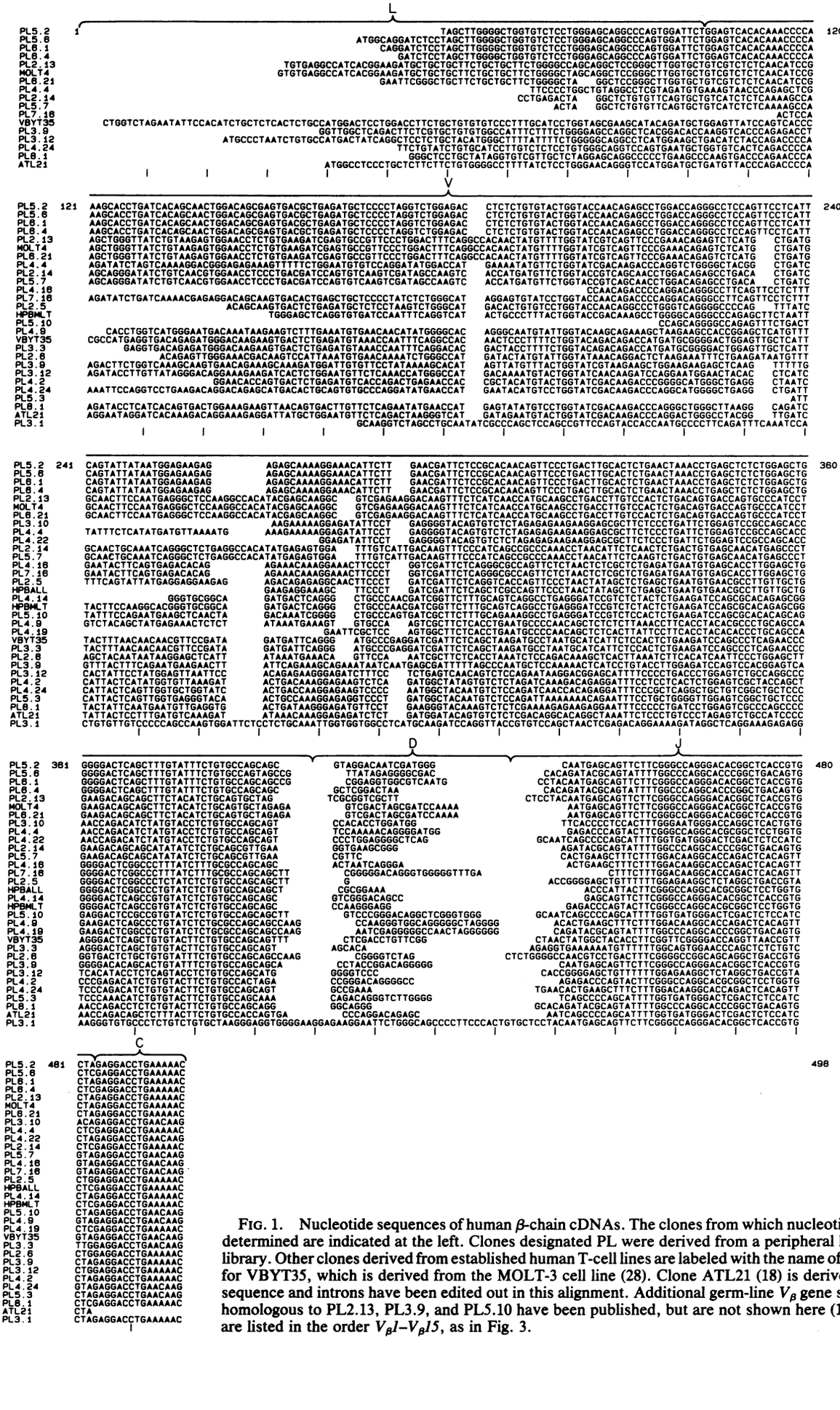

FIG. 1. Nucleotide sequences of human β-chain cDNAs. The clones from which nucleotide sequences were determined are indicated at the left. Clones designated PL were derived from a peripheral lymphocyte cDNA library. Other clones derived from established human T-cell lines are labeled with the name of the cell line except for VBYT35, which is derived from the MOLT-3 cell line (28). Clone ATL21 (18) is derived from a genomic sequence and introns have been edited out in this alignment. Additional germ-line V_β gene segment sequences homologous to PL2.13, PL3.9, and PL5.10 have been published, but are not shown here (18). The sequences are listed in the order $V_\beta 1$–$V_\beta 15$, as in Fig. 3.

Isolation of β-Chain cDNA Clones. An *Ava* I/*Eco*RV C region fragment of clone pYT35 (28) was used to screen 2 × 10^5 plaques from the peripheral lymphocyte cDNA library. Phage DNA was isolated on DEAE columns (29), and cDNA inserts were sized on 0.7% agarose gels. Inserts >1 kb long were subcloned into M13 mp18.

DNA Sequencing. Sequences corresponding to the V, D, and J regions of the β-chain cDNA clones were determined by the method of Sanger *et al.* (30) using 5′-[[α-^{35}S]thio]triphosphate (31). Sequencing reactions were primed either with the 17-base M13 universal sequencing primer, or with an 18-base oligonucleotide, 5′ TTCTGATGGCTCAAACAC 3′, which is complementary to a region of perfect homology near the 5′ end of the human $C_\beta 1$ and $C_\beta 2$ genes. All sequences were determined on both strands with the exception of clones PL4.9 and PL8.1.

Southern Blot Analyses. V_β-specific probes were made by subcloning restriction fragments from V regions of β-chain cDNA clones into appropriately digested pUC12 plasmid. Probes were labeled with [α-^{32}P]dNTPs by nick-translation (32). Blots were prepared on Zeta-Probe nylon membranes (Bio-Rad) and hybridized according to the procedures of Gatti *et al.* (33) or Reed and Mann (34). Final washes were for 30 min at 65°C in 1× SSC/0.1% NaDodSO$_4$ (1× SSC is 0.15 M NaCl/0.015 sodium citrate).

RESULTS AND DISCUSSION

Repertoire of Expressed Human V_β Genes. The nucleotide sequences of 27 β-chain cDNA clones derived from a normal human peripheral blood lymphocyte cDNA library are compared with five published human β-chain cDNA sequences derived from leukemic cell lines in Fig. 1 (18, 28, 35–37). There are 22 unique V_β gene segments represented among these sequences. Six of these V_β gene segments appear more than once in our analysis, and three more have been published elsewhere (18). If we assume that these V_β gene segments represent a random sampling of expressed human V_β gene segments, and that all V_β gene segments are expressed with essentially the same probability, then a statistical calculation allows us to estimate the size of the human expressed V_β gene repertoire (15, 16). This calculation suggests that the expressed human V_β gene segment pool size is <59 at the 95% confidence level. The human V_β gene pool appears to be intermediate between the estimated sizes of the mouse V_β repertoire of ≈25 genes (15–17) and the mouse V_α repertoire of >100 (38) and substantially less than estimated pool sizes for V_H or V_κ region genes, which contain on the order of 200–1000 genes (19–21).

Clone PL3.1 does not contain a V_β gene and probably is a partial copy of a J–C transcript, since its sequence is colinear with germ-line DNA 5′ to its J_β sequence (39). In light of the high frequency of multiple β-chain rearrangements in T cells, it is surprising that apparently none of the sequences from our peripheral blood lymphocyte library is derived from nonproductive transcripts.

V_β Gene Subfamilies. The percentage similarity of the nucleotide sequences of the 22 different human V_β gene segments range from 31% to 99%. From this information, we can divide the characterized human V_β gene segments into 15 different subfamilies by the criteria of 75% or more shared nucleotide homology (23). We have isolated members from 14 of these 15 families. The subfamilies have been designated $V_\beta 1$–$V_\beta 15$, in keeping with the nomenclature proposed by Barth *et al.* (15). In this nomenclature, individual members of subfamilies are identified by a digit following a decimal point. For example, the $V_\beta 7$ subfamily contains at least two members, $V_\beta 7.1$ (PL4.9) and $V_\beta 7.2$ (PL4.19).

To gain an estimate of the sizes of these human V_β gene subfamilies, we used V_β gene-specific probes, subcloned from cDNA clones representing the various subfamilies, to probe germ-line DNA on genomic blots (Fig. 2). Two different restriction digests of human genomic DNA were hybridized with each probe to establish a correspondence between numbers of hybridizing bands and numbers of genes. The method yields only minimum estimates of subfamily sizes. For example, probes derived from short cDNA clones, such as $V_\beta 6.2$, sometimes fail to hybridize to band numbers commensurate with the known number of subfamily members (18). In addition some probes, such as $V_\beta 11$, hybridize to very large genomic DNA fragments, which could contain multiple V_β gene segments. Taking into account both nucleotide sequence and Southern blot data, the 15 V_β gene subfamilies that we have described consist of one subfamily with six members ($V_\beta 6$) (18), one with five members ($V_\beta 8$) (5), one with four members ($V_\beta 13$), two with three members ($V_\beta 5$, $V_\beta 14$), four with two members ($V_\beta 2$, $V_\beta 4$, $V_\beta 7$, $V_\beta 15$), and six

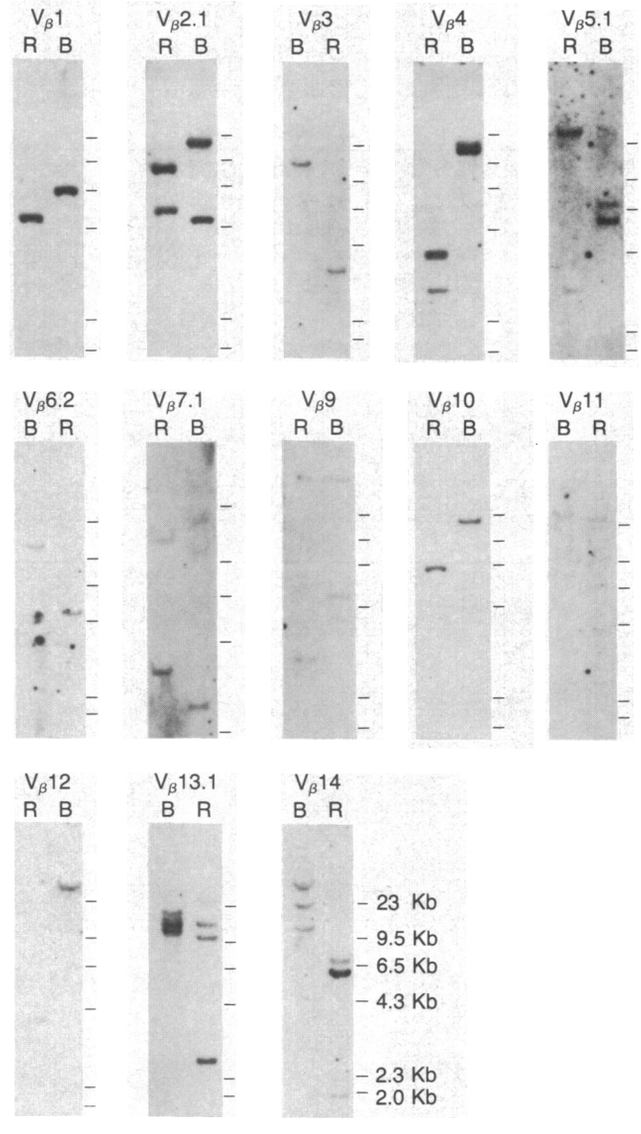

FIG. 2. Southern blot analysis of human germ-line DNA with V_β probes. HeLa cell DNA was digested with *Eco*RI (R) and *Bam*HI (B), electrophoresed through 1% agarose gels, blotted to nylon membranes, and hybridized with the indicated V_β gene segment probes. The distance of migration of size standards is indicated. The $V_\beta 8$ and $V_\beta 15$ subfamilies have been excluded but are published elsewhere (5, 18). The $V_\beta 6$ subfamily has been demonstrated to have six members in a separate analysis (18). Under less stringent washing conditions, 18 additional genes are detected with these probes (data not shown).

with one member ($V_\beta 1$, $V_\beta 3$, $V_\beta 9$, $V_\beta 10$, $V_\beta 11$, $V_\beta 12$). Therefore, human V_β gene subfamilies are more frequently multimembered than their mouse V_β counterparts.

Variability Among V_β Gene Segments. Fig. 3 shows an alignment of 22 unique human V_β gene segment amino acid sequences with 16 mouse V_β sequences. These V_β gene segments are highly heterogeneous when their amino acid sequences are compared either within or between species. The amino acid homology between human V_β genes of different subfamilies ranges from 14% to 63%. This maximum range of variability, 86%, is slightly higher than that for human V_H genes at 76%. Despite this variability, there are 16 positions in V_β regions at which one amino acid occurs >75% of the time. These positions have been indicated in Fig. 3. Six of these residues are nearly invariant in a comparison of V_β, V_α, V_L, and V_H regions. These positions are those of structural importance in immunoglobulins such as the cysteines that form the intrachain disulfide bond in the V region or residues involved in interchain interactions. Twelve of the 16 conserved amino acids in V_β gene segments are conserved at the 75% level in at least one other class of V region; 10 in V_α and V_L and eight in V_H. Four positions, Leu-19, His-27, Ser-96, and Ser-97, are conserved exclusively in V_β regions. This conservation of structurally important amino acids between V_β, V_α, V_L, and V_H is consistent with the idea that immunoglobulin and T-cell receptor V regions share similar tertiary structures. Structural predictions for β-pleated sheets and turns (40, 41) also support the hypothesis of a similarity in three-dimensional structure (data not shown).

Based on a comparison of translated mouse V_β gene segment sequences, it has been suggested that V_β gene segments contain multiple unique hypervariable regions not observed in comparisons of immunoglobulin V gene segments (17). The existence of such unique hypervariable regions in T-cell receptor chains would be particularly significant in light of the requirement for T-cell receptor to recognize not only foreign antigen but also polymorphic MHC restricting element. Fig. 4 shows a variability plot of translated V_β gene segment sequences utilizing the algorithm of Wu and Kabat (24) and analyzing the sequences as aligned in Fig. 3. This variability plot shows evidence only for the two classically defined hypervariable regions (24) and the third region as defined by Capra and Kehoe (25). Its peaks of variability closely match those seen in a variability plot generated from the sequences of 18 randomly chosen human V_H genes (15). The fourth hypervariable region is located at the D–J boundary.

Somatic Hypermutation. Comparisons of identical murine V_β genes used in different cDNA clones (15–17), or comparisons of germ-line and rearranged human V_β genes (18), reveal only a limited number of nucleotide differences, which may reflect either a low level of somatic hypermutation or polymorphism. A comparison of identical V_β genes that we have analyzed confirms this observation. We found no differences in comparisons of three examples of $V_\beta 3$, two examples of $V_\beta 5$, two examples of $V_\beta 6.1$, and two examples of $V_\beta 6.2$. We did find a single silent nucleotide substitution in one of four examples of $V_\beta 1$, a single replacement nucleotide difference between two examples of $V_\beta 4$, and a single replacement nucleotide substitution between two examples of $V_\beta 2.1$. The frequency with which these differences occur is substantially less than that found in comparisons of immunoglobulin V genes where somatic hypermutation may create up to 3% sequence difference (13). We believe that these relatively minor differences reflect genetic polymorphisms in the human population, although somatic hypermutation cannot be excluded as a cause. However, somatic hypermutation in V_L or V_H genes usually leads to multiple substitutions that are concentrated presumably by selection in the hypervariable regions. The diversity observed here is limited in each case to single nucleotide changes that are randomly distributed. Furthermore, since most of the sequences we have examined are derived from peripheral lymphocytes, we presume that they include mature T cells, which can be reasonably compared to the mature B cells in which somatic hypermutation is observed.

***D*$_\beta$ and *J*$_\beta$ Gene Segment Contributions to Diversity.** We have been able to determine the identity of the D_β sequence ($D_\beta 1$ or $D_\beta 2$) used in 24 of the 31 human D region-containing cDNA clones we have examined. The 3′ end of the D_β regions in individual cDNA clones was determined by reference to germ-line J_β and D_β sequences (7, 39). The 3′ ends of the V gene segments were determined by comparison to germ-line

FIG. 3. Amino acid sequences of murine and human V_β genes. Human (h) and murine (m) V_β gene-segment nucleotide sequences were translated and aligned to maximize homology at the amino acid level. Amino acids conserved at the 75% level are indicated by asterisks. $V_\beta 2.1$ and $V_\beta 2.2$ differ only in the leader sequence and hence appear identical in this comparison. Amino acids are identified by the single-letter code.

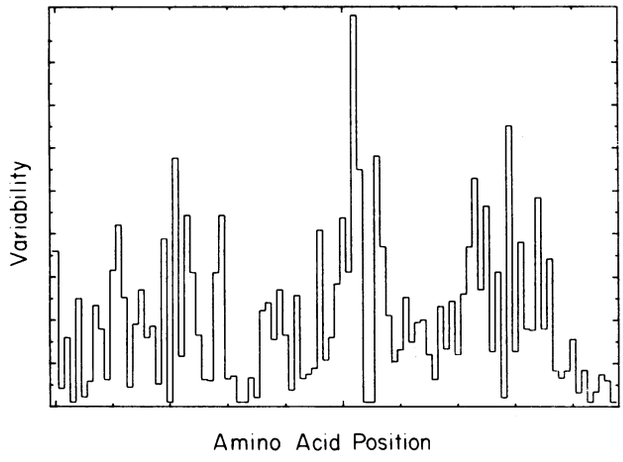

FIG. 4. Variability plot of V_β gene segments. Variability has been calculated and plotted for each position in the translated human and murine V_β gene segments aligned in Fig. 3. Variability is defined as the number of different amino acids that occur at a particular position divided by the frequency of the most common amino acid at that position.

V_β and D_β sequences when available (18). In other cases, it was defined as the next nucleotide after the last shared nucleotide in multiple clones using the same V_β gene segment or V_β gene segments of the same subfamily. The D regions defined by these criteria ranged from 1 to 24 nucleotides long. At least one clone, PL4.19, appears to contain a $D_\beta 1.1$–$D_\beta 2.1$ fusion (L.A.P., unpublished observations).

There are several important contributing factors to diversification at the V_β–J_β junction. First, there is extensive use of junctional and N-region diversity, which, in some clones, nearly obscures the identity of the germ-line D_β segment. A second factor is that independently derived nucleotide sequences for the germ-line $D_\beta 2.1$ gene segment vary at one position (7, 39). We have found cDNA clones that conform to each of these sequences, indicating that this may be a common polymorphism in human populations. In all but three cases, assignment of D_β identity was made based on the presence of five or more nucleotides colinear with germ-line D_β segment sequence.

As would be expected from random usage, $D_\beta 1.1$ rearranges equally to either J_β gene cluster. However, $D_\beta 1.1$ (15/24) is used slightly more often than $D_\beta 2.1$ (9/24). While this may reflect the fact that the $D_\beta 2.1$ sequence contains a stop codon in one of its reading frames, there is no obvious bias against the use of this blocked reading frame in $D_\beta 2.1$-containing clones. As in mouse D_β gene segment usage (42), we have observed essentially random usage of both D_β gene segments in all three reading frames. In the case of $D_\beta 1.1$, reading frames 1, 2, and 3 are used 4/15, 6/15, and 5/15 times, respectively. In $D_\beta 2.1$-containing clones, the first and second reading frames are used 3/9 and 4/9 times, respectively. The third reading frame of $D_\beta 2.1$, which contains the in-frame termination codon, is used 2/9 times, and in both cases the termination codon has been deleted in the joining process.

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