Structure and Diversity of the Human T-Cell Receptor β-Chain Variable Region Genes

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In order to characterize the variability of the expressed human T-cell receptor (TCR) β-chain repertoire and contrast this variability to the known murine β-chain repertoire, 15 independent complementary DNA (cDNA) clones containing TCR β-chain variable region (V_β) genes were isolated from a human tonsil cDNA library. The nucleotide and derived amino acid sequences of these 15 V_β genes were analyzed together with 7 previously defined sequences. Fifteen different human V_β genes could be identified from 22 independent sequences. By means of DNA hybridization and sequence homology comparisons, it was possible to group these 15 genes into ten distinct V_β subfamilies, each containing from one to seven members. Minimal polymorphism was noted between individuals, except in multimember subfamilies. The amino acid sequences of these genes contain conserved amino acids that are also shared by murine TCR V_β genes and immunoglobulins; no features were found that distinguish human V_β genes from their murine counterparts. Evaluation of secondary structure showed that maximum variability coincides with generally hydrophilic portions of the amino acid sequence, while specific hydrophobic regions were conserved in all V_β genes examined.

THE T-CELL RECEPTOR (TCR) IS A heterodimer of disulfide-linked α and β chains, each composed of an amino-terminal variable (V) region and a carboxyl-terminal constant (C) region (*1–3*). Antigen recognition by the TCR is presumed to occur in the V region (*4*). Unlike immunoglobulins, however, the TCR recognizes antigen only in the context of molecules encoded by the major histocompatibility complex (MHC), a phenomenon known as MHC restriction (*5, 6*). At present, the mechanisms responsible for MHC restriction are unknown. Murine TCR V_β genes are known to consist of a relatively small number of one- to three-member subfamilies, which show very little polymorphism among various strains of inbred mice (*7–10*). These V_β gene segments somatically rearrange to diversity (D) and joining (J) gene segments to create a functional variable region, a process that magnifies the germline-encoded variability.

The structure and diversity of murine TCR V_β genes have been well defined (*7–10*). To similarly characterize the structure and diversity of human TCR V_β genes, we examined 15 V_β-containing complementary DNA's (cDNA's) and compared them with the murine (*7–10*) and human (*11–15*) V_β genes published to date. We find that human V_β genes show many similarities to their murine homologs in both amino acid sequence and predicted secondary structure. While the size and organization of the germline repertoire of human V_β genes is unknown, we estimate the expressed repertoire to be about 50 genes.

A cDNA library was constructed from a human tonsil, and a portion of the primary, unamplified library was screened with a murine TCR C_β probe; C_β-positive cDNA clones were isolated, and their nucleotide sequences were determined. A total of 15 independent V_β-containing cDNA's were obtained; their nucleotide sequences are presented in Fig. 1 along with those of seven V_β genes previously defined by others. The sequences are grouped by homology so that duplicate isolates of a single V_β gene and related members of different subfamilies can be more easily recognized. It is not clear whether highly homologous isolates represent distinct members of the same subfamily or are actually allelic variants of the same gene isolated repeatedly from a polymorphic V_β gene pool. In this report, genes that are not identical will be presented as distinct V_β genes.

Seven of the 22 nucleotide sequences in Fig. 1 are identical in their V_β coding region to previous V_β isolates (the sequences of these isolates are represented by dashes in Fig. 1). All duplicate isolates possess distinct junctional sequences and therefore represent independent, repeated usage of a single V_β gene. Interestingly, duplicate pairs of identical V_β genes were isolated both from the same source (ph 16 and 79, ph 7 and 34, and ph 26 and 29, from our tonsil cDNA library) and from different individuals (ATL 12-2 and ph 5, MT1-1 and Molt 4, YT35 and ph 11, and ATL 2-1 and ph 32). Our ph 7 and ph 34 genes are 99% homologous to the published Molt 4 and MT1-1 genes (Fig. 2) and may represent either allelic polymorphism or recently duplicated, yet distinct, genes; genomic Southern blot analysis indicates that ph 7 is part of a two-member V_β subfamily (Fig. 3). Similarly, nine nucleotide differences were found between a germline human V_β gene and the YT35 cDNA (*14*). Our ph 8 and ph 11 cDNA's (Fig. 1) differ at the same nine nucleotides and correspond exactly to both of these published genes; genomic Southern blot analysis indicates that YT35 is part of a five-member V_β subfamily (*14*).

In summary, the 22 sequences presented in Fig. 1 include seven genes isolated in duplicate and eight genes isolated once, so that 15 distinct V_β genes have been defined. Thus, 32% (7/22) of V_β isolates to date involve repeated usage of previously defined V_β genes. If one assumes that all V_β genes are utilized equally, a statistical estimate can be made of the likely number of V_β genes that may be present in the human genome (*9, 16, 17*). The maximum likelihood estimate of the number of distinct V_β gene segments (n) is 25, which was obtained by determining the value of n that maximized the probability for 15 distinct gene segments and a total number of 22. A 95% one-sided confidence bound of $n = 52$ was obtained by determining the smallest value of n for which the probability of observing at most 15 different segments among 22 segments examined was less than 0.05. If ph 34 and Molt 4 are allelic variants and not distinct genes, as discussed above, then the number of distinct gene segments is 14 and estimates of n will be slightly lower (*18*).

It is possible that our cDNA library is not representative of human V_β usage in general since it was derived from a single human tonsil, in which antigenic stimulation and clonal proliferation may have resulted in the relative overexpression of particular V_β genes. However, when our sequences are pooled with those previously identified, eight independent sources of V_β sequences are represented. Within the limitations of our sampling, we conclude that the expressed human V_β repertoire is less than 50 to 60 genes. This calculation will underestimate the actual number of human V_β genes if V_β usage is nonrandom in human peripheral lymphoid tissue; nonrandom V_β usage has already been demonstrated in the murine splenic T-cell population (*10*). Nevertheless, these data indicate that the human V_β repertoire is likely to be larger than the murine V_β repertoire of < 30 genes (*7–10*) but smaller than the immunoglobulin heavy-chain variable region (V_H) repertoire of 250 to 300 genes (*19*).

Clonal diversity in human β-chain variable regions is generated in a fashion identical to the murine V_β region, in which the

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diversity of a limited number of germline gene elements is enhanced by a flexible somatic recombination scheme. Toyonaga *et al.* (*20*) have shown that there are six J-region gene segments associated with $C_\beta 1$ and seven J regions associated with $C_\beta 2$, with at least one diversity gene segment located 5′ of each cluster of J_β elements (*20*). Analysis of the junctional regions of the cDNA sequences in Fig. 1 shows that diversity in the recombination event is apparently greater than would be expected

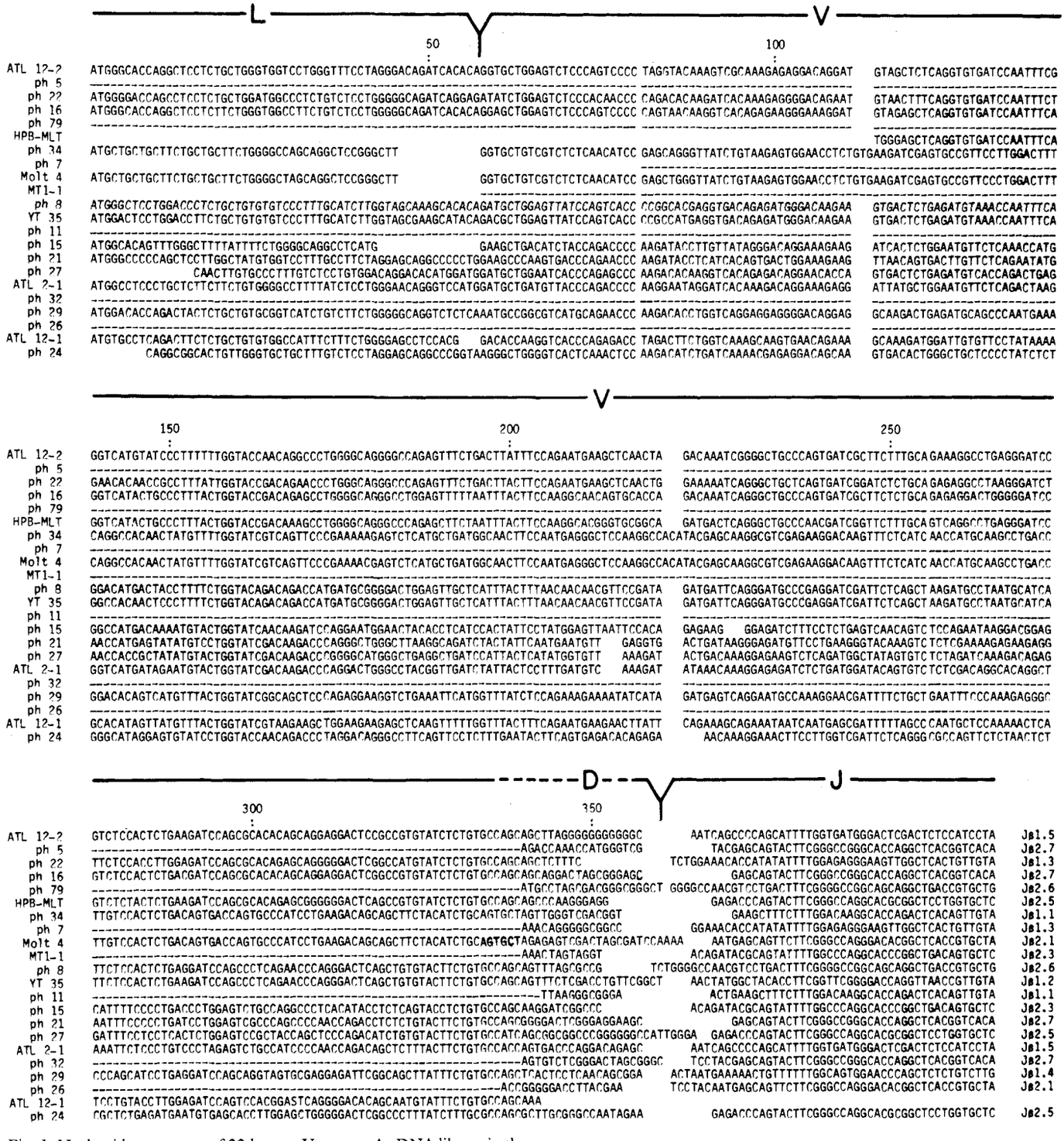


Fig. 1. Nucleotide sequences of 22 human V_β genes. A cDNA library in the λgt10 cloning vector was constructed with polyadenylated RNA from a human tonsil (*32*, *33*). The library was screened with a murine C_β probe; DNA from positive clones was subcloned into pUC 19 or M13mp 18. Nucleotide sequences were determined by either the method of Maxam and Gilbert (*34*) or the method of Sanger *et al.* (*35*), as modified by Biggin *et al.* (*36*). A synthetic primer complementary to C_β was used to sequence from C_β into the V region. Sequences begin within the leader; the J_β elements are aligned separately and are identified according to Toyonaga *et al.* (*20*). Gaps have been introduced to maximize homology. Genes that are identical to a previously defined sequence have their nucleotide sequences represented by dashes, indicating base identity with the nucleotide sequence immediately above the dash. Sources of V_β sequences are as follows: ph 5 to ph 79, this work; MT1-1, ATL 2-1, ATL 12-1, ATL 12-2 (*11*); YT35 (*15*); Molt 4 (*13*); and HPB-MLT (*12*).

	ATL 12-2	ph 22	ph 16	HPB-MLT	ph 34	Molt 4	ph 8	YT 35	ph 15	ph 21	ph 27	ATL 2-1	ph 29	ATL 12-1	ph 24
ATL 12-2	—	71	76	73	32	32	56	58	27	28	36	29	48	38	48
ph 22	82	—	65	66	31	31	53	54	30	33	40	36	45	42	45
ph 16	81	79	—	78	36	36	59	59	38	30	45	34	46	38	44
HPB-MLT	78	76	86	—	30	30	58	60	30	26	34	30	48	40	42
ph 34	49	51	49	52	—	98	31	32	31	27	32	35	29	28	28
Molt 4	49	51	49	52	99	—	31	31	31	27	32	35	29	28	28
ph 8	67	68	70	70	48	48	—	97	32	38	44	39	51	40	44
YT35	67	68	70	70	48	48	98	—	32	38	44	39	50	38	45
ph 15	52	51	50	45	46	46	54	53	—	53	57	54	33	27	29
ph 21	49	54	52	51	49	49	55	54	70	—	55	57	32	32	34
ph 27	55	57	57	51	52	52	59	58	66	69	—	60	32	29	39
ATL 2-1	52	55	54	47	52	52	56	56	69	70	70	—	32	35	39
ph 29	56	59	57	56	50	50	60	59	52	52	56	50	—	47	40
ATL 12-1	55	62	56	59	49	49	57	57	44	52	53	50	62	—	41
ph 24	55	58	56	55	45	45	53	54	51	45	53	53	57	56	—

Fig. 2. Homology matrix of 15 human V_β gene segments. Numbers above the diagonal indicate the percentage of homology between the sequences indicated on the *x*- and *y*-axes when compared at the amino acid level; numbers below the diagonal show percentage homology at the nucleotide level. V_β coding sequences, excluding leader segments, are compared.

solely from a flexible joining process. Putative D regions, defined as sequence not encoded by genomic J or identifiable V elements, vary significantly in length (Fig. 1). These junctional regions differ widely in the number of nucleotides originating from the two known germline D_β segment genes. The sequence of clone ph 16 has 15 putative "D-region" nucleotides, 13 of which appear to be encoded by $D_\beta 2.1$, whereas ph 34 and YT35 have only 3 of 12 nucleotides and 3 of 15 nucleotides, respectively, of recognizable germline origin. We conclude that either more pronounced N-region diversification occurs during the human V_β-D_β-J_β recombination event than has generally been seen in murine β-chain cDNA's (*7–9*) or additional unidentified human germline D_β elements exist.

Comparison of germline and rearranged human or murine V_β genes suggests that somatic hypermutation is not significantly involved in the generation of TCR β-chain diversity (*7–9, 11*). T cells utilizing the same germline genes generally express identical V_β gene segments in sequenced cDNA's. Duplicate isolates of the same V_β genes are identical in seven cases examined here, supporting the conclusion that somatic hypermutation is a rare event.

Comparisons of the 15 different human V_β gene sequences to each other show eight genes that are greater than 75% homologous (Fig. 2). These genes should cross-hybridize on genomic Southern blot analysis and therefore define related V_β subfamilies. V_β genes ATL 12-2 (ph 5), ph 22, ph 16 (ph 79), and HPB MLT; V_β genes YT35 (ph 11) and ph 8; and V_β genes MT1-1 (Molt 4) and ph 34 (ph 7) define three distinct multimember subfamilies. The remaining genes are all <70% homologous to other isolates and define seven additional subfamilies. These seven subfamilies may also prove to have multiple members. In summary, 15 different V_β gene sequences examined define ten V_β subfamilies.

The number of bands visualized on a Southern blot of genomic DNA hybridized with a V_β probe corresponds approximately to the number of related genes within a subfamily. When representative V_β probes from each subfamily are used in Southern blot analysis and the number of bands is counted, a more realistic estimate of the human V_β repertoire can be made. At least seven bands are observed when a $V_\beta$ph 5 (ATL 12-2) probe is used (Fig. 3). Similarly, YT35 (our ph 11) shows five bands (*14*), while $V_\beta$ph 7, $V_\beta$ph 15, and $V_\beta$ph 32 each show two bands (Fig. 3 and not shown). $V_\beta$ph 21, $V_\beta$ph 27, and $V_\beta$ph 29 each show a single band (not shown). Thus, representative V_β probes from eight separate subfamilies identify 21 bands on genomic Southern blots. Interestingly, 13 of the 21 putative V_β genes from these eight subfamilies have already been cloned and their sequences determined (Fig. 1). The human V_β gene family therefore appears to be composed of at least ten subfamilies, each containing one to seven members. When the composition of the human V_β gene family is compared to the murine V_β gene family (*7–10*), a similar small number of subfamilies is found. However, human V_β gene subfamilies are often composed of multiple members while murine V_β genes mostly belong to single member subfamilies. Therefore, while both the human and mouse species possess a similar number of V_β subfamilies, humans seem to have more than twice the number of actual V_β genes. It is not clear why human V_β subfamilies often expand to contain multiple members while their murine counterparts do not.

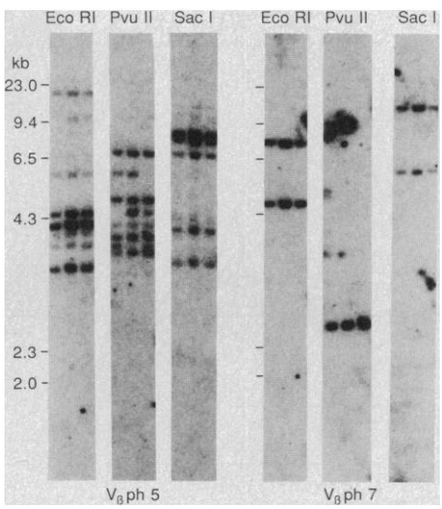


Fig. 3. Genomic Southern blot of peripheral-blood-leukocyte DNA from three individuals. DNA (10 μg) (*37*) was digested with Eco RI, Pvu II, and Sac I as indicated, separated on 0.8% agarose gels, and transferred to nitrocellulose (*38*). DNA on the filters was hybridized to nick-translated V_β-specific probes (specific activity: 1×10^8 to 3×10^8 cpm/μg) at 68°C for >12 hours, under standard conditions (*39*). Filters were washed in 0.30*M* NaCl/0.03*M* sodium citrate at 68°C and autoradiographed. Positions of marker fragments from Hind III–digested λ DNA are indicated on the left.

Little polymorphism has been seen in V_β gene subfamilies among various strains of inbred mice (*7–10*). It is not known to what extent polymorphism exists in the overall V_β gene family in the outbred human population. However, in a limited survey, we note the existence of both seemingly nonpolymorphic and highly polymorphic V_β gene subfamilies among the genes presented here. A $V_\beta$ph 7 probe (Fig. 3) shows no restriction fragment length polymorphism (RFLP) in a genomic Southern blot analysis of three individuals and thus represents a class of relatively nonpolymorphic V_β genes, similar to the pattern observed for most V_β genes among the inbred mouse population. In contrast, RFLP's are clearly seen among individuals in the $V_\beta$ph 5 (ATL 12-2) multimember subfamily (Fig. 3). Increased polymorphism in multimember subfamilies may result from gene exchanges between highly homologous but independent genes, as has been suggested to occur for immunoglobulins (*21*). Whether the RFLP's correlate with actual V_β sequence polymorphism is not known.

Homology between members of different subfamilies ranges from 26 to 60% (Fig. 2). Comparison of the amino acid sequences of human V_β genes (Fig. 4) with the amino acid sequences of available murine V_β genes shows no difference in conserved residues; most of the residues conserved in V_β genes

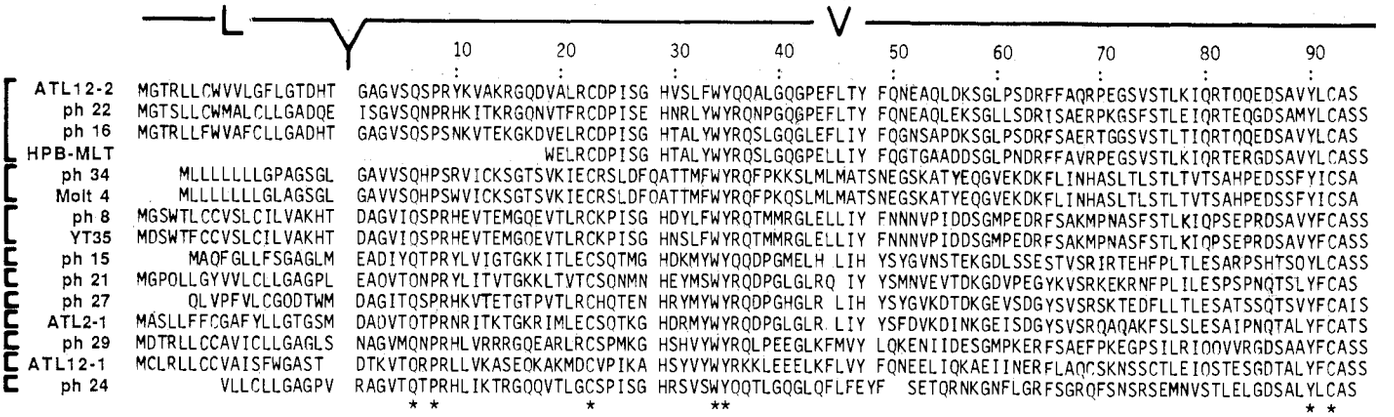
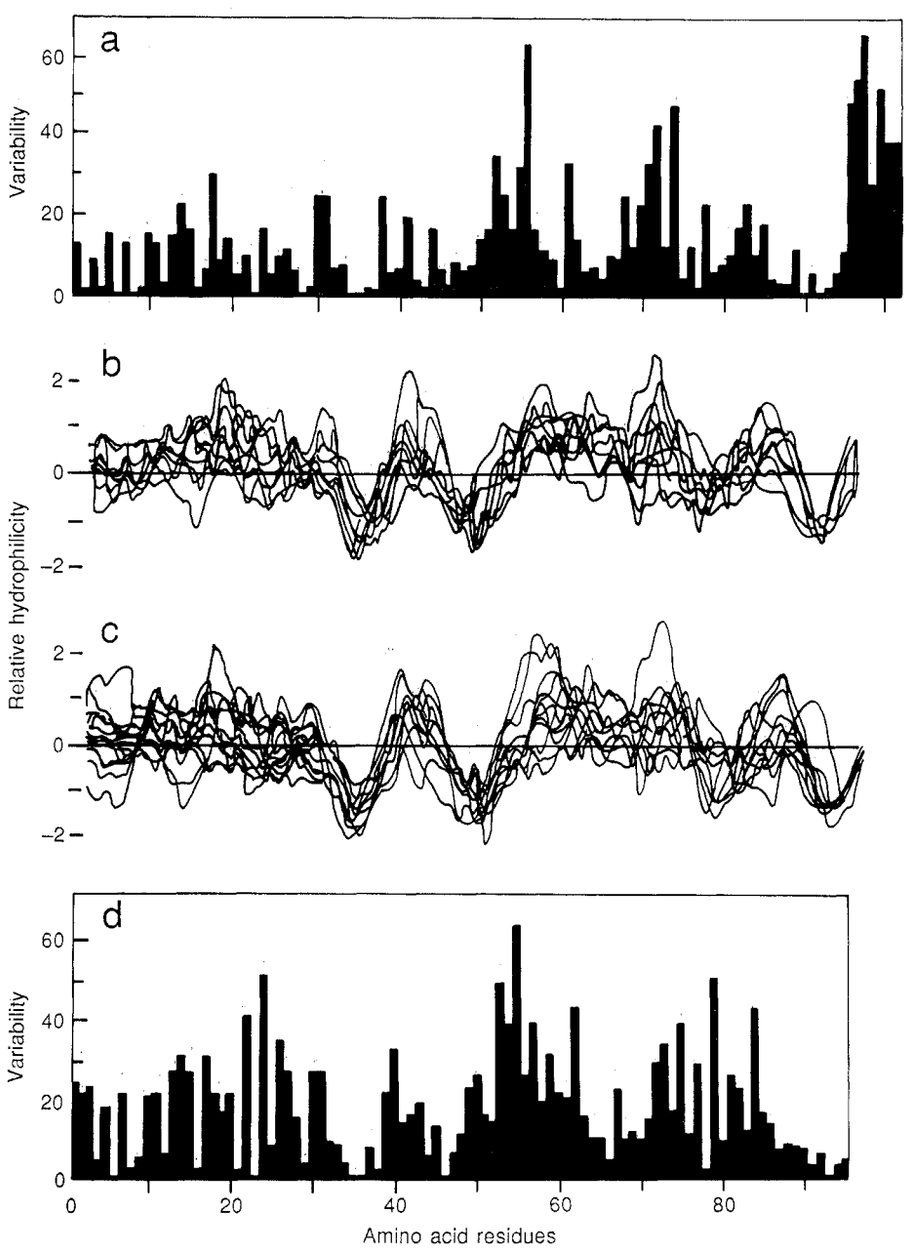


Fig. 4. Translated amino acid sequences of 15 different human V_β genes. The nucleotide sequences of the 15 distinct V_β sequences of Fig. 1 were translated and aligned to maximize amino acid sequence homology. Putative leader sequences are indicated and aligned separately. Asterisks identify invariant residues. Brackets group the 15 sequences into ten subfamilies according to homology (Fig. 2).

Fig. 5. Variability and hydrophilicity analysis of human and murine V_β genes. Variability analysis was done according to Wu and Kabat (*25*). Relative hydrophilicity was determined from the algorithm of Hopp and Woods (*29*). Amino acid position is plotted on the *x*-axis. (a) Variability plot of the 15 human V_β genes translated in Fig. 3, including the D to J junctions. (b) Superimposed relative hydrophilicity plots of the following 12 human V_β genes: ph 5, ph 22, ph 16, ph 34, ph 8, ph 15, ph 21, ph 27, ph 32, ph 29, V_βATL 12-1, ph 24. (c) Superimposed relative hydrophilicity plots of the following 12 murine V_β genes [nomenclature according to Barth *et al.* (*8*) and Behlke *et al.* (*10*)]: $V_\beta1$, $V_\beta2$, $V_\beta3$, $V_\beta4$, $V_\beta5.1$, $V_\beta6$, $V_\beta7$, $V_\beta8.1$, $V_\beta9$, $V_\beta11$, $V_\beta12$, and $V_\beta14$. (d) Variability plot of 19 murine V_β genes; D to J junctions are not included.



are also present in TCR V_α genes (*22–24*) and in the variable region genes of immunoglobulins (*25*). Despite a xenogeneic difference between their MHC-restriction elements, no common features could be defined that would distinguish human V_β genes from their murine counterparts. Variability among the 15 human V_β genes was analyzed in a Wu-Kabat variability plot (*25*) (Fig. 5a). In addition to variability encoded within the V_β gene segments, significant variability is introduced at the V_β-D_β-J_β junction. For comparison, a Wu-Kabat variability analysis of 19 murine V_β gene segments is included (Fig. 5d).

The hypervariable regions of immunoglobulins are believed to be involved in antigen interaction (*26–28*). By analogy, it would be predicted that MHC/antigen specificity should reside in the hypervariable regions of the TCR. Regions directly involved in ligand interaction should be in the hydrophilic regions of the molecule. Twelve human V_β genes and 12 murine V_β genes were analyzed with the hydrophilicity algorithm of Hopp and Woods (*29*). As shown

in Fig. 5, b and c, the areas of highest variability on the Wu-Kabat analysis correspond, in general, with hydrophilic regions of the molecule, consistent with these regions being available to interact with ligand. In addition, the hydrophilicity plot shows conservation of several hydrophobic regions, which are thought to be involved in the formation of a common tertiary structure shared among all TCR chains as well as members of the immunoglobulin supergene family (*4, 30*).

Pairwise comparison of the 15 human V_β genes in Figs. 1 and 4 with known murine V_β sequences does not present any clear picture of allelic evolutionary relationships. For example, human V_β genes ATL 12-2, ph 22, ph 16, HPB-MLT, ph 8, YT35, and ph 29 are all more homologous to the murine V_β11 gene than any other known murine V_β gene (54 to 68% at the amino acid level, 64 to 78% at the nucleotide level). Some of these genes are actually more homologous to the murine gene than they are to each other. Thus seven different human V_β genes representing three different subfamilies all show significant homology to the same murine V_β gene. In other cases, significant homology is found in only a single murine-human pairwise combination. ATL 12-1 is most homologous to the murine V_β3 gene (54% amino acid, 66% nucleotide), ph 24 is most homologous to the murine V_β5.2 gene (55% amino acid, 65% nucleotide), and ph 34 and Molt 4 are most homologous to the murine V_β15 gene (47% amino acid, 64% nucleotide). When pairwise comparisons between the most homologous murine and human V_β genes are aligned together, amino acid substitutions are seen to occur more often in hydrophilic regions of the molecule, and the frequency of these substitutions roughly parallels the plot of V_β variability in general. In these cases, therefore, evolution of V_β alleles between species is similar to the evolution of nonallelic V_β genes within a species, with changes being concentrated in the hydrophilic regions of the molecule.

Note added in proof: Similar results have been obtained by Kimura *et al.* (*31*).

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Replication of the B19 Parvovirus in Human Bone Marrow Cell Cultures

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The B19 parvovirus is responsible for at least three human diseases. The virus was successfully propagated in suspension cultures of human erythroid bone marrow from patients with hemolytic anemias; release of newly synthesized virus into the supernatants of infected cultures was observed. This culture system allowed study at a molecular level of events associated with the B19 life cycle. The B19 parvovirus replicated through high molecular weight intermediate forms, linked through a terminal hairpin structure. B19 replication in vitro was highly dependent on the erythropoietic content of cultures and on addition of the hormone erythropoietin.

THE B19 PARVOVIRUS WAS DISCOVERED in the sera of normal blood donors in 1975 (*1*) and subsequently was identified as the causative agent of (i) transient aplastic crisis of hemolytic disease (*2*), (ii) the common childhood exanthem called fifth disease (*3*), and (iii) a polyarthralgia syndrome in normal adults (*4*). In addition, B19 parvovirus may be responsible for some cases of hydrops fetalis (*5*). The genome of the B19 virus consists of about 5.4 kb of single-stranded DNA (*6–8*), which encodes at least two capsid proteins of about 84 and 58 kD (*9, 10*). Genetic studies have been hampered by the inability to propagate the B19 parvovirus in vitro. Even though B19-containing sera are potent inhibitors of colony formation by bone marrow erythroid progenitor cells (*11*), clonogenic assays are inadequate for either maintenance or molecular study of the B19 virus. We report here successful propagation of B19 parvovirus in a tissue culture system by means of fresh human bone marrow cells.

Serum containing B19 parvovirus (Minor II, 60 μg of B19 DNA per milliliter) was obtained from a patient with sickle cell disease and transient aplastic crisis during a recent B19 epidemic (*12*). It was adsorbed to erythroid bone marrow mononuclear cells that had been obtained with informed consent from patients with hemolytic anemias; the infected cells were then grown in standard growth medium in the presence of the erythroid cell–specific hormone erythropoietin. Virus addition only slightly de-

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