HUMAN T CELL RECEPTOR V_β GENE POLYMORPHISM

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The human T cell receptor for antigen is composed of two chains, α and β, which form a disulfide-linked heterodimer on the surface of mature T cells (1). The ability of this receptor to recognize foreign antigens in the context of MHC antigens is critical to the immune response (2). The genes encoding the α and β chains of the human T cell have been cloned, and their genomic organization has been determined (3–8). Similar studies have also been carried out on murine T cell receptor genes (for review see references 9, 10).

The human T cell receptor β chain locus is located at chromosomal position 7q32–35 (11–14). The β chain genes are assembled in the DNA of developing T cells by a series of specific chromosomal rearrangement events that join several noncontiguously encoded gene segments. Within the locus there are two highly homologous constant region genes, $C_\beta 1$ and $C_\beta 2$. A cluster of six or seven joining (J_β) gene segments is located to the 5' side of each C_β gene. One diversity (D_β) gene segment is located 5' to each J_β gene segment cluster (7). The variable (V_β) gene segments are presumed to lie at some unknown distance to the 5' side of the region containing the two D_β-J_β-C_β gene segment clusters.

We (15) and others (16, 17) have estimated the number of expressed V_β gene segments by statistical analysis of the frequency of their occurrence in cDNA libraries generated from either peripheral blood or tonsils. The currently identified V_β gene segments can be grouped into 18 subfamilies based on the criteria of ≥75% shared nucleotide homology (18). Probes representative of these subfamilies have been hybridized to genomic DNA derived from one or a few individuals to ascertain how many total germline V_β gene segments exist in individual subfamilies (15–17).

To more accurately determine the size and characteristics of the human germline V_β gene repertoire, we have examined non–T cell genomic DNA from ~100 individuals with probes representing 14 different V_β gene segment subfamilies containing ~48 gene segments. We have identified restriction fragment length polymorphisms associated with 12 of 14 subfamilies and have demonstrated linkage disequilibrium between the alleles of some V_β loci. To date, no V_β gene segment appears to exhibit more than two alleles. For those polymorphisms with alleles that are frequent in the population, we demonstrated segregation. The haplotypes identified by allotyping at linked V_β loci should prove useful in understanding the genetics of T cell immune responses and in the

investigation of genetically transmitted disease susceptibilities or immune dys-functions.

Materials and Methods

DNA Samples. High-molecular-weight DNA was isolated from a panel of 30 lympho-blastic cell lines (LG series) derived from the offspring of consanguineous marriages and homozygous for class I and class II MHC antigens (19). DNA samples from lymphoblastoid cell lines representing 69 additional unrelated individuals were supplied through the courtesy of CEPH (Centre pour l'Etudé du Polymorphisme Humain), a Paris-based gene-mapping consortium. We also examined DNAs derived from some additional cell lines and from some tissue samples. Rearrangement of the C_β genes was not observed in any of these DNA samples.

Probes. Nucleic acid probes corresponding to members of human V_β gene segment subfamilies $V_\beta 1$ through $V_\beta 14$ were isolated from subclones described by Concannon et al. (15). For subfamilies 5 and 7 we used probes corresponding to two different subfamily members. Gel-purified inserts containing V_β-specific sequences were labeled by random priming with α-[^{32}P]triphosphates (20) to specific activities ranging from 5×10^8 to 1×10^9 cpm/μg and were used without further purification.

Southern Blots. Restriction digests of genomic DNAs were carried out under conditions specified by the enzyme manufacturers. In the case of DNAs from the LG cell lines, the completeness of digestion was monitored by testing the ability of a 1-μg aliquot of the reaction mixture to digest an additional 1 μg of λ DNA simultaneously. 5–10 μg of DNA was loaded per lane on gels to be transferred. Blots were prepared either by the method of Gatti et al. (21) or Reed and Mann (22) on Zeta-Probe nylon membranes (Bio-Rad Laboratories, Richmond, CA). The same blots were used for all V_β gene hybridizations so that the results of each round of hybridization could be directly compared. Some blots were useful for hybridizations with more than 20 probes.

Hybridizations were carried out for 12–15 h at 37°C in 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate), 0.02 M sodium phosphate, pH 6.7, 100 μg/ml sheared denatured salmon sperm DNA, 0.5% nonfat powdered dry milk, 10% dextran sulfate, 1% SDS, and 1–2 ng/ml of probe. Filters were washed in 2× SSC and 0.1% SDS at 60°C and exposed to Kodak XAR-5 X-ray film for 12 to 120 h. Probe was removed for subsequent rounds of hybridization by washing twice in boiling .01× SSC for 15 min on a room temperature rocker platform.

Results and Discussion

Relative Sizes of Human V_β Gene Segment Subfamilies. We have hybridized probes representing each of the human T cell receptor V_β gene subfamilies, $V_\beta 1$ through $V_\beta 14$, to germline DNA from ~100 unrelated individuals. For 30 of these individuals (the LG series cell lines), this analysis was carried out with four different restriction enzymes. This allowed us to estimate the size of the various V_β gene segment subfamilies with greater accuracy because it avoided problems of interpretation caused by polymorphism in outbred human populations. As shown in Fig. 1, the 14 V_β subfamilies contain at least 48 V_β gene segments. As in the mouse (23–25), the subfamilies are small in size, the largest containing seven members; but unlike the mouse, fewer are single membered.

By statistical analysis of V_β gene representation in cDNA libraries, we have previously estimated the expressed human V_β gene repertoire to be ≤ 59 genes with 95% confidence (15). The 48 genes we have now identified by hybridization approach this number and yet do not include three additional members of the $V_\beta 6$ subfamily described by Ikuta et al. (26) and those members contained in subfamilies $V_\beta 15$, $V_\beta 16$, $V_\beta 17$, and $V_\beta 18$ (a total of at least eight members) (16,
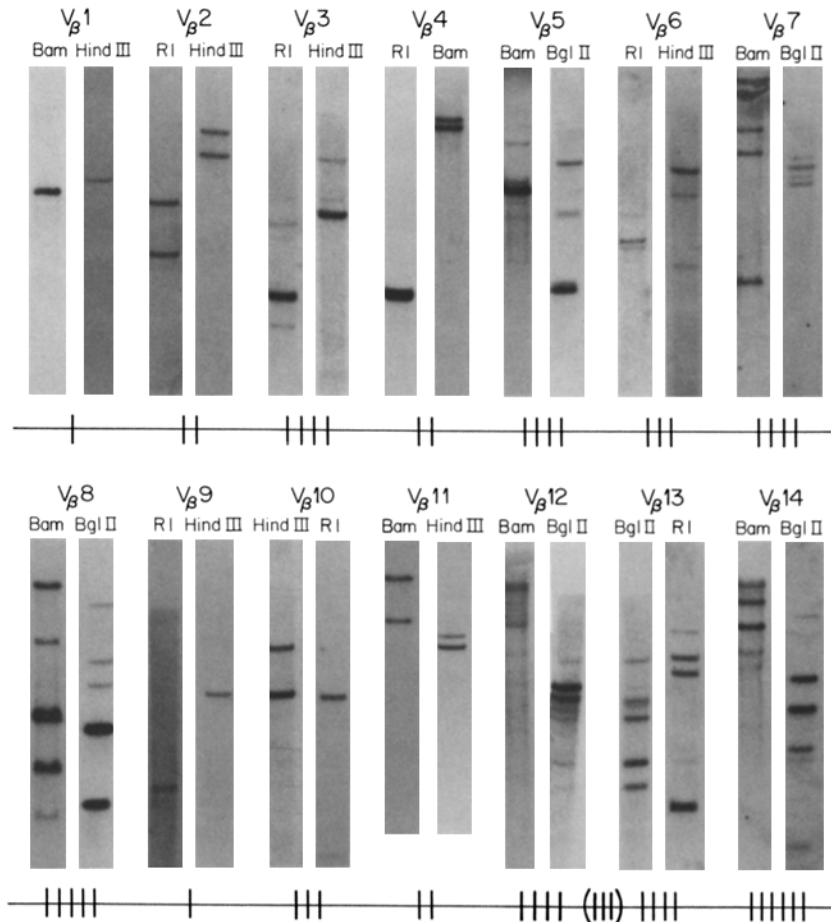
FIGURE 1.   Southern blot analyses displaying the most common restriction patterns for T cell receptor V_β gene segment subfamilies 1 through 14. Two different digests are shown for each subfamily. Vertical lines indicate the estimated numbers of gene segments within individual subfamilies. In some cases, estimates of subfamily sizes were made based on additional hybridization data not shown. Subfamilies $V_\beta12$ and $V_\beta13$ appear to share common members as indicated by nucleotide sequence data (15, 17).

17). Therefore, it is likely that the total number of V_β genes in the human genome will exceed that which can be estimated by statistical analysis of expressed genes. Some of the V_β gene segments detected by hybridization will correspond to pseudogenes that cannot be productively rearranged, or are not transcribed in populations of mature T cells (27). Therefore, these gene segments would not be represented in cDNA libraries from peripheral lymphocytes.

Variation in the V_β Gene Segment Repertoire Size.   Analysis of inbred mouse strains by hybridization with murine V_β-specific probes has indicated that the size of the V_β gene repertoire can vary. Certain inbred strains, SJL, C57L, and C57BR, have been shown to lack V_β gene segments present in most other inbred mouse strains (28). For example, SJL mice lack approximately half of the normal repertoire of V_β gene segments (28). We were interested in determining if similar variations occur in humans and if so, at what frequency in the general population.
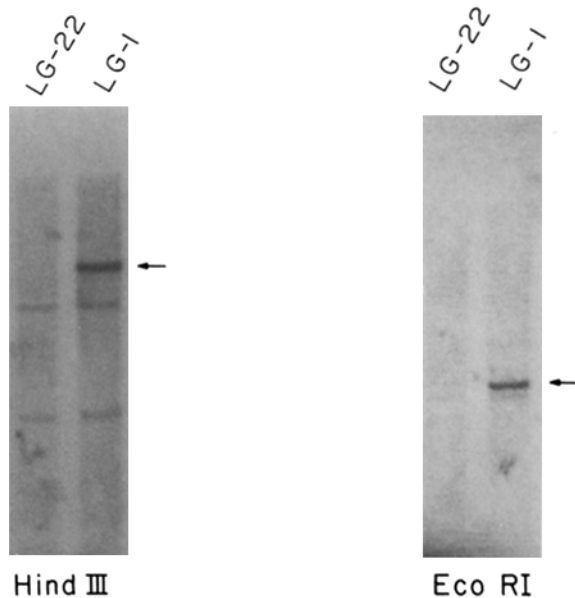
FIGURE 2. Southern blot analyses demonstrating the homozygous deletion of a member of the $V_\beta6$ subfamily. LG-22 DNA lacks the strongly hybridizing band corresponding to the $V_\beta6.2$ gene segment indicated by the arrow in LG-1 DNA. This band was present in DNA samples from 99 of 100 individuals tested.

Hind III   Eco RI

One way to demonstrate variation in repertoire size unambiguously is to identify individuals that are homozygous for deletions of certain V_β gene segments, as was the case for inbred mice. The DNAs derived from consanguineous individuals (LG series) are particularly suited to this analysis because they can be reasonably expected to be homozygous for a greater proportion of their genes. In screening the DNA from ~100 unrelated individuals with V_β gene segment probes, we identified only one who had such a deletion, represented by the cell line LG-22. This cell line, derived from a Venezuelan Indian of the genetically isolated Waraos tribe (29), lacks a single member of the $V_\beta6$ subfamily. Based on the homology of this fragment to a $V_\beta6.2$ probe in other DNA samples, we believe that it is the $V_\beta6.2$ gene segment that is deleted. The deletion is revealed with all four restriction enzymes tested and does not affect any other V_β gene within the $V_\beta6$ subfamily or in the 13 other subfamilies we have tested. Fig. 2 shows the results of hybridization of a $V_\beta6.2$ probe to LG-22 DNA and DNA from an unrelated individual, LG-1, digested with two different restriction enzymes.

A second type of variation in the V_β gene segment repertoire occurs in the cell line LG-41 and affects the $V_\beta3$ subfamily. We have defined the $V_\beta3$ subfamily as containing four members, although the member that is least homologous to our probe hybridizes only very weakly under routine conditions used in our laboratory. However, when LG-41 DNA is digested with a variety of different restriction enzymes, this band hybridizes significantly more strongly to the probe, appearing as the second most homologous member of the subfamily. Fig. 3 shows a comparison of DNA from the cell lines LG-41 and LG-42 digested with two different restriction enzymes and hybridized to a $V_\beta3$ probe. The increase in hybridization signal seen with LG-41 DNA is not accompanied by a shift in restriction fragment size with any of the enzymes we have tested, and sequence differences responsible for this effect are localized by this analysis to a region of
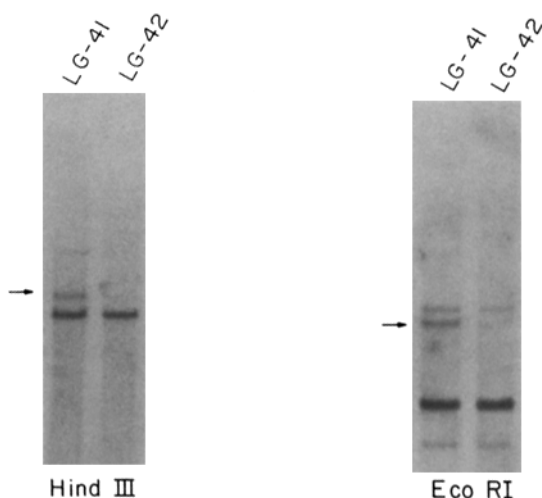
FIGURE 3. Southern blot analyses depicting increased hybridization of a member of the $V_\beta 3$ subfamily in LG-41 DNA. One member of the $V_\beta 3$ subfamily, indicated by the arrow, displays a reproducibility increased intensity of hybridization exclusively in DNA from the LG-41 cell line.

6.0 kb. The hybridization of other $V_\beta 3$ subfamily members acts as an internal comparison standard, ruling out the possibility that this is a DNA concentration effect. Gene duplication could cause this effect; however, the increase in hybridization intensity is great enough that it would require multiple duplications. Restriction mapping data indicates that the duplication could not alter the hybridization pattern of other V_β gene segments. The most likely explanation for this result is a gene conversion event that has made the faintly hybridizing gene segment more homologous to the probe without altering the positions of flanking restriction enzyme sites. This was the only variation of this type in the 100 individuals sampled.

Polymorphism Associated with V_β Gene Segments.   To further assess the amount of polymorphism associated with V_β gene segments, we hybridized DNA from each of the 30 LG cell lines digested with four different restriction enzymes to probes for each of the $V_\beta 1$ through $V_\beta 14$ gene segment subfamilies. Table I describes the probe/enzyme combinations that detected polymorphic differences between individuals within this panel. These polymorphisms were specific for the restriction enzyme used in the analysis and recurring patterns were observed with DNAs from different individuals. We found such polymorphisms associated with 12 of the 14 V_β gene segment subfamilies tested.

Polymorphic restriction sites for several different enzymes were detected with some probes, (e.g., $V_\beta 2$ with Hind III and Bgl II; $V_\beta 8$ with Bam HI, Hind III, and Bgl II; $V_\beta 11$ with Bam HI and Bgl II; and $V_\beta 12$ with Bam HI and Hind III). Three polymorphisms (see below) involved alleles that were relatively common in our panel. For most V_β probes, however, we observed one predominant hybridization pattern with only a few individuals displaying a second pattern. Because of the infrequency of these variant forms in our small panel, we were unable in many cases to observe the minimal three hybridization patterns required to define a biallelic polymorphism (i.e., AA, AB, and BB) or to find informative families with which to demonstrate segregation of these less common polymorphisms. For these polymorphisms, we have indicated in Table II the frequencies of the various hybridization patterns observed for each of the polymorphic V_β gene segment subfamilies.

TABLE I

Incidence of Polymorphism in Human V_β Gene Segment Subfamilies

Subfamily	Eco RI	Bam HI	Hind III	Bgl II
$V_\beta 1$	−	−	+	−
$V_\beta 2$	−	−	+	+
$V_\beta 3$	+	+	+	+
$V_\beta 4$	+	−	−	−
$V_\beta 5$	−	−	−	+
$V_\beta 6$	+	+	+	+
$V_\beta 7$	−	−	+	+
$V_\beta 8$	−	+	+	+
$V_\beta 9$	−	−	−	−
$V_\beta 10$	−	−	−	−
$V_\beta 11$	−	+	−	+
$V_\beta 12$	−	+	+	−
$V_\beta 13$	−	−	−	+
$V_\beta 14$	−	−	+	−

(+) The probe/enzyme combinations that detected polymorphism when DNA from 30 unrelated individuals was digested with four different restriction enzymes and blotted with probes representing 14 V_β gene segment subfamilies. Polymorphism in the $V_\beta 3$ and $V_\beta 6$ subfamilies are specific to single cell lines, LG-22 and LG-41, as described in the text. (−) Probe/enzyme combinations that failed to detect polymorphism.

Two of the probe/enzyme combinations ($V_\beta 8$/Bam HI and $V_\beta 12$/Bam HI) appeared to detect the same polymorphic Bam HI site. Subsequent restriction mapping of a cosmid clone that hybridized to both the $V_\beta 8$ and $V_\beta 12$ probes indicated that these probes were hybridizing to V_β gene segments that flanked a single polymorphic Bam HI restriction site (Concannon, P., unpublished observations).

Segregation of V_β Gene Polymorphisms. Two of the polymorphisms we identified that displayed the minimal three hybridization patterns consistent with the segregation of two alleles at each locus ($V_\beta 8$/Bam HI and $V_\beta 11$/Bam HI) were tested for segregation. We hybridized the $V_\beta 8$ and $V_\beta 11$ probes sequentially to Bam HI–digested genomic DNA from members of several three-generation families, one of which is shown in Fig. 4. To increase the informativeness of markers in this genomic area, we also tested the segregation of a previously reported polymorphism detected with a C_β probe (30) in Bgl II–digested DNA from this family. The $V_\beta 8$ probe hybridized to two or three bands when washed under high stringency (1× SSC/0.1% SDS at 65°C), a constant band of 3.3 kb containing the $V_\beta 8.2$ gene segment, and a polymorphic band of either 23 or 2.0 kb containing the $V_\beta 8.1$ gene segment. In our analysis of 100 unrelated individuals, the 23-kb allele occurred at a frequency of 46.4% and the 2.0-kb allele at a frequency of 53.5%. The $V_\beta 11$ probe hybridized to a constant band of 12 kb and polymorphic bands of either 25 and/or 20 kb in 100 unrelated individuals. The 25-kb allele occurred at a frequency of 47.4% and the 20-kb allele at a frequency of 52.6%. The C_β probe hybridized to bands of either 10 or 9.0 kb in the same panel of individuals. The 10-kb allele occurred at a frequency of 55.9% and the 9-kb allele at a frequency of 44.1%. A diagram of this family and the

TABLE II

Polymorphic Hybridization Patterns Detected in LG Cell Lines with V_β Gene Segment Probes

Cell Line	DR	$V_{\beta}1$ Hind III	$V_{\beta}2$ Hind III	$V_{\beta}2$ Bgl II	$V_{\beta}3$ Eco RI	$V_{\beta}4$ Eco RI	$V_{\beta}5$ Bgl II	$V_{\beta}6$ Eco RI	$V_{\beta}7$ Hind III	$V_{\beta}7$ Bgl II	$V_{\beta}8$ Bam HI	$V_{\beta}8$ Hind III	$V_{\beta}8$ Bgl II	$V_{\beta}11$ Bam HI	$V_{\beta}11$ Bgl II	$V_{\beta}12$ Bam HI	$V_{\beta}12$ Hind III	$V_{\beta}13$ Bgl II	$V_{\beta}14$ Hind III
LG-2	1													B			C		
LG-15	1						B							B	B		C	B	
LG-30	1	ND	ND								B					B	C		
LG-6	1					C								C					
LG-7	2					C					C			C			C		
LG-26	2	ND	ND						ND		C	ND		C		C	ND		ND
LG-22	2		B					B			C						B		
LG-1	4						B				C					C	B		
LG-25	4	ND	ND										B	B			ND		
LG-39	4								B					B			B		
LG-19	5										B					B	ND		B
LG-50	5										C			C		C	B		
LG-35	5										B		B			B	B		
LG-32	6									ND		ND		B			B		B
LG-49	6	ND	ND	ND			ND		ND	ND		ND	ND	B	ND		ND	ND	ND
LG-28	6	B									C			C		C	ND	B	B
LG-18	6	ND	ND						ND			ND		C			ND	B	ND
LG-10	7								ND		B						B	B	
LG-36	7																		
LG-51	7													B			ND		
LG-5	3						B							B	B			B	
LG-14	3			B							C	B	B	C		C	C		
LG-27	3													B					
LG-52	3										B						B	ND	
LG-41	8					B				B	B			C			B		
LG-42	8									B	B						B	B	
LG-53	9				B	B		B			B						B		
LG-13	10				B						B						B		B
LG-9	11	ND	ND						ND			ND		B			ND		ND
LG-37	?										B						B	B	B

Vertical columns contain data relevant to specific probe/enzyme combinations. Horizontal columns indicate results for individual LG cell lines. A blank space indicates that the most common hybridization pattern for that particular probe/enzyme combination was seen (i.e., the A allele). B and C indicate less and least frequent patterns, respectively. ND, a combination in which unambiguous identification of pattern was not possible.

results obtained by hybridization with $V_{\beta}8$, $V_{\beta}11$, and C_{β} probes is shown in Fig. 4.

Linkage Disequilibrium Between Alleles at the $V_{\beta}8$ and $V_{\beta}11$ Gene Loci. The haplotypes created by the three probes described above, $V_{\beta}8$, $V_{\beta}11$, and C_{β}, are potentially very informative markers for following genetic segregation because the alleles at each locus are approximately equal in frequency, and hence one might expect to see each of the theoretically possible nine haplotypes in outbred human populations with high frequencies. However, analysis of 70 unrelated individuals homozygous for at least one of the three loci indicated that certain alleles at the $V_{\beta}8$ and $V_{\beta}11$ loci were preferentially associated and hence were in linkage disequilibrium. 52 of these subjects were doubly homozygous, allowing the assignment of haplotypes. Fig. 5 indicates both the distribution of haplotypes expected in this sampling, based on allelic frequencies at these three loci, and the actual distribution observed. The observed distribution of haplotypes is highly biased towards those haplotypes in which the $V_{\beta}8$ 2.0-kb allele is associated with the $V_{\beta}11$ 25-kb allele and those in which the $V_{\beta}8$ 23-kb allele is associated with the $V_{\beta}11$ 20-kb allele ($p < 0.001$).

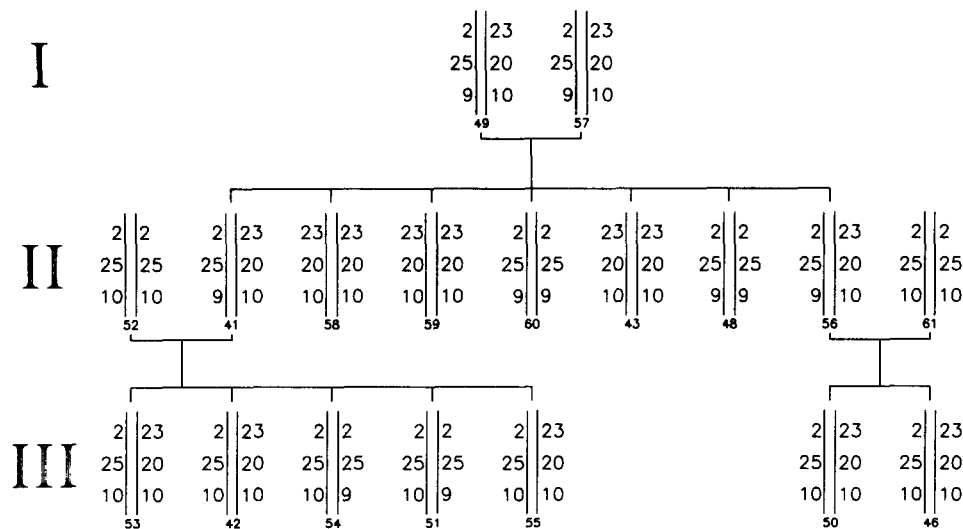That alleles at the $V_{\beta}8$ and $V_{\beta}11$ loci were in strong linkage disequilibrium is

I

2	23	2	23
25	20	25	20
9	10	9	10
49		57	

II

2	2	2	23	23	23	23	23	2	2	23	23	2	2	2	23	2	2
25	25	25	20	20	20	20	20	25	25	20	20	25	25	25	20	25	25
10	10	9	10	10	10	10	10	9	9	10	10	9	9	9	10	10	10
52		41		58		59		60		43		48		56		61	

III

2	23	2	23	2	2	2	2	2	23	2	23	2	23
25	20	25	20	25	25	25	25	25	20	25	20	25	20
10	10	10	10	10	9	10	9	10	10	10	10	10	10
53		42		54		51		55		50		46	

FIGURE 4. Segregation of polymorphic V_β and C_β alleles. Each chromosome indicates the alleles detected at the $V_\beta8$, $V_\beta11$, and C_β loci ordered top to bottom, respectively. Numbers below schematic chromosomes are for subject identification.

further evidenced by the fact that of the 30 individuals who were homozygous for the 2.0-kb allele at the $V_\beta8$ locus, 26 (87%) were also homozygous for the 25-kb allele at the $V_\beta11$ locus, compared with an expected 7 (23%) individuals ($p < 0.001$). Conversely, of 24 individuals who were homozygous for the 23-kb allele at the $V_\beta8$ locus, 22 (92%) were homozygous for the 20-kb allele at the $V_\beta11$ locus, compared with an expected number of 7 (30%) ($p < 0.001$). This nonrandom distribution also mildly affected alleles at the C_β locus. For example, only one individual homozygous for the 9-kb allele at C_β was seen among those people who were homozygous for the 23-kb allele at the $V_\beta8$ locus, instead of the 5 expected, and of the 12 individuals in our panel who were homozygous for the 9-kb allele at the C_β locus, 6 were homozygous for the 25-kb allele at the $V_\beta11$ locus as opposed to the 3 expected. However, these latter figures were not statistically significant ($0.1 > p > 0.05$).

There are several possible explanations for this disequilibrium. First, the loci could be very close from a genetic standpoint, either because of close physical linkage, or recent generation of alleles. Our finding of allelic frequencies approaching 50% in a variety of populations in a panel of unrelated individuals containing representatives of a number of different populations, and the finding of the same alleles among these representatives, argues that this effect is probably not due to recent mutation. Second, there could be a suppression of recombination in the region containing these loci that would cause them to appear linked. Studies of the recombination rates between polymorphic V_β gene segment alleles and alleles at flanking loci could test this possibility. Third, there could be some selective pressure favoring the existence of certain haplotypes over others. Preliminary results suggest that at least the first explanation plays a role in the observed disequilibrium since the $V_\beta8$, $V_\beta11$, and C_β loci all appear to map to a restriction fragment of <600 kb in size (Lai, E., P. Concannon, and L. Hood, manuscript in preparation).
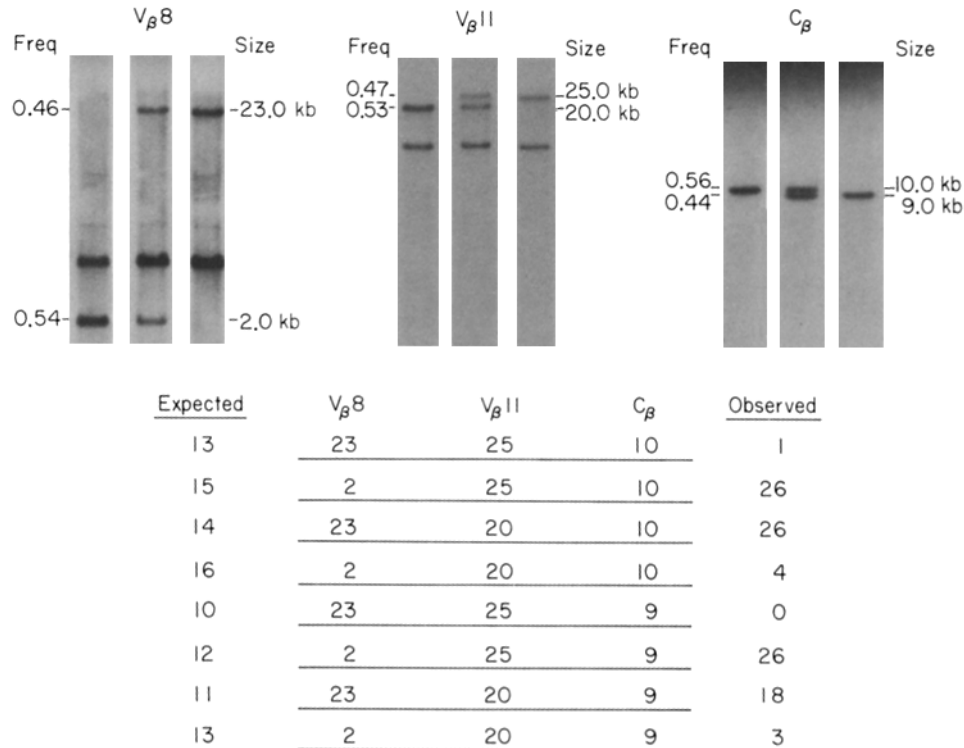
Expected	$V_\beta 8$	$V_\beta 11$	C_β	Observed
13	23	25	10	1
15	2	25	10	26
14	23	20	10	26
16	2	20	10	4
10	23	25	9	0
12	2	25	9	26
11	23	20	9	18
13	2	20	9	3

FIGURE 5. Linkage disequilibrium between the alleles at the $V_\beta 8$ and $V_\beta 11$ loci. Southern blots display the hybridization patterns for both homozygotes and the heterozygote at each locus. Allelic frequencies are indicated to the left. Fragment size is indicated to the right. The distribution of haplotypes in a population of 52 unrelated individuals generated by genotyping at the $V_\beta 8$, $V_\beta 11$, and C_β loci are indicated below. The distribution of haplotypes expected is calculated from the allelic frequencies above.

Despite the fact that many of these linked polymorphic alleles were of low frequency in our panel, among the 30 individuals we examined, 27 different haplotypes were identified. These haplotypes should prove useful in determining if there are associations between the T cell receptor locus and inherited disease involving the immune system. Indeed, in a recent study, Hoover et al. (31) were able to demonstrate a putative association between alleles at the C_β locus and insulin-dependent diabetes mellitus. The V_β gene polymorphisms we have described should be very useful in strengthening this, and possibly other disease associations.

Summary

Southern blot hybridizations with human T cell receptor V_β gene probes were used to determine the sizes of the various V_β gene subfamilies. An analysis of DNA samples from 100 unrelated individuals identified a single individual who lacked one V_β gene segment. A second individual had an apparently different repertoire of V_β gene segments in one subfamily, as assayed by hybridization, possibly due to a gene conversion event. An analysis with four restriction enzymes of DNA from 30 consanguineous donors detected restriction fragment length polymorphisms associated with 12 of 14 V_β gene segment subfamilies examined.

In an analysis of DNAs from a large panel of unrelated individuals, some alleles at these loci were found to be in linkage disequilibrium, indicating a potentially close physical linkage. The segregation of three polymorphisms, two associated with V_β gene segment loci and one associated with the C_β genes, was compatible with Mendelian inheritance, and demonstrated that highly informative haplotypes could be generated. The high degree of polymorphism observed in the human T cell receptor β chain complex should allow exploration of possible associations between T cell receptor genes and inherited diseases involving the immune system.

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