THE Vγ LOCUS OF THE HUMAN T CELL RECEPTOR γ GENE

Repertoire Polymorphism of the First Variable Gene Segment Subgroup

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T cell rearranging γ genes encode a protein expressed on the cell surface of a small subset of thymocytes and peripheral T lymphocytes (1–6). These genes rearrange early during thymic maturation. The protein encoded by the T cell γ genes is associated with the product of another newly recognized rearranging gene, δ, in a complex associated with the CD3 molecules (7–10). It is probable that the CD3-γ/δ complex functions as a receptor, but its ligand has not yet been determined (11). The structure of the γ gene has been extensively studied, demonstrating the presence of 15 variable segments located 5′ to junctional segments and constant regions. The variable segments are grouped in four subgroups. The VγII, VγIII, and VγIV subgroups contain only one member while the VγI subgroup contains nine segments, four of which are pseudogenes (12–15). The precise localization of Vγ5 with regard to the VγI subgroup remains unknown. During a recent study of Vγ rearrangements in some leukemic cells (15), we have observed an absence of Vγ4 and Vγ5 segments that is not explicable by any mechanism of rearrangment. This observation prompted us to investigate this DNA region further by cloning and sequencing and to study EBV cell lines from normal donor families in order to search for a possible polymorphism of the VγI repertoire. In the present study, we show that this polymorphism is indeed frequent and may therefore reflect an evolutionary gene replication event. We also report some other polymorphisms occurring in the VγI locus.

Materials and Methods

DNA Samples and Strategy of the Study.　72 DNA samples from EBV-transformed lymphoblastoid cell lines were studied. No γ gene rearrangement was detected in these DNA samples using a Jγ probe (data not shown). These DNA samples were supplied by the Centre d'Etude du Polymorphisme Humain (Paris, France) and were obtained from both parents of

36 healthy Caucasian families. Restriction analysis of the DNA samples was performed using Eco RI, Hind III, Taq I, and Sac I restriction enzymes. A probe recognizing the first subgroup was used. Polymorphisms were observed in Eco RI and Taq I digests and were further analyzed by the study of segregation in 10 informative families from the 36 families collected by the CEPH. DNA samples from both parents, all children (average n = 8), and in the majority of cases, all four grandparents were studied.

Southern Analysis. DNA extraction was performed by the usual methods (16). High molecular weight DNAs were digested with restriction enzymes, fractionated on agarose gels and blotted onto nylon membranes (Hybond N, Amersham International, Amersham, UK). Filters were hybridized with the appropriate probes, ^{32}P labeled by the method of Feinberg and Volgelstein (17). Prehybridization and hybridization were performed in 50% formamide containing media at 42°C.

Probes. The Jγ probe (18) is a 700-bp Eco RI Hind III fragment from the clone M13H60 (19). The VγI probe, a 1.2-kb Sac I fragment containing Vγ3 and isolated from λSH4 (12), recognizes all VγI segments. VγII probe (12) is a Pst I-Taq I fragment of the λKγ20 clone (19). VγIII probe (13) is a 0.6-kb Pst I-Eco RI insert (pRPO.6) that contains the 5' region of Vγ10. The VγIV probe is a Rsa I-Rsa I fragment of our P41 clone (15).

Genomic Libraries, Establishment of the Restriction Maps, and Analysis of DNA Sequence. Part of the VγI subgroup was cloned from the B16 and DS6 genomic EMBL 3 libraries, after screening with the VγI probe. As described elsewhere (20), B16 DNA was obtained from a case of acute lymphoblastic leukemia of B cell lineage, which demonstrated a ψVγC rearrangement on one allele and deletion of the Vγ-Jγ-Cγ locus on the other. The construction of the B16 library is described elsewhere (15). A clone containing a functional Vγ5-Jγ1 rearrangement obtained by screening of the DS6 library with a Jγ probe was also studied. This library was established from a γ/δ receptor–expressing T cell clone (21, 22). The restriction maps of the phage clones were analyzed by a series of double digestions and DNA sequences were determined according to Sanger (23). Relevant fragments were subcloned in mp11 and mp10. The universal M13 primer and specific oligonucleotides were used.

Results

A Frequent Repertoire Polymorphism Involving the Vγ4 and Vγ5 Gene Segments. 72 DNA samples, digested by Eco RI, were hybridized with the VγI probe. In all but one sample, eight bands were observed, each corresponding to one or two Vγ segments (13). The two 3.1-kb and 2.5-kb fragments corresponding to the 3' and 5' part of the Vγ4 segment were lacking in one DNA sample. No additional band or increased intensity of normal sized bands was observed in this sample. Furthermore, the intensity of the 3.6-kb fragment that corresponds to a comigration of Vγ5 and Vγ7 was obviously decreased (Fig. 1 *E*, lane *3*). Previous analysis of a leukemic case with Vγ5 rearrangement (15) showed that the majority of this band constituted Vγ5. We hypothesized that this particular VγI pattern corresponded to lack of a DNA fragment containing the Vγ4 and Vγ5 segments. A Taq I digest was analyzed to strengthen this hypothesis. As shown in Fig. 1 *T*, lane *3*, the two 6.2- and 4.4-kb fragments corresponding to the Vγ4 and Vγ5 segments, respectively, were lacking. It was thus probable that Vγ4 and Vγ5 were lacking in both chromosomes. To confirm this, we determined the size of the Bam HI segment that contains all VγI segments. The comparison of the previously mentioned DNA sample with a DNA containing the Vγ4 and Vγ5 segments on both chromosomes showed that the lack of Vγ4 and Vγ5 was associated with a decrease in fragment size of ~10 kb (i.e., 31 kb vs. 41 kb; Fig. 1 *B*). As the distance between two consecutive VγI segments is ~5-kb (12), this result could easily be explained by the absence of a DNA fragment encompassing Vγ4 and Vγ5.
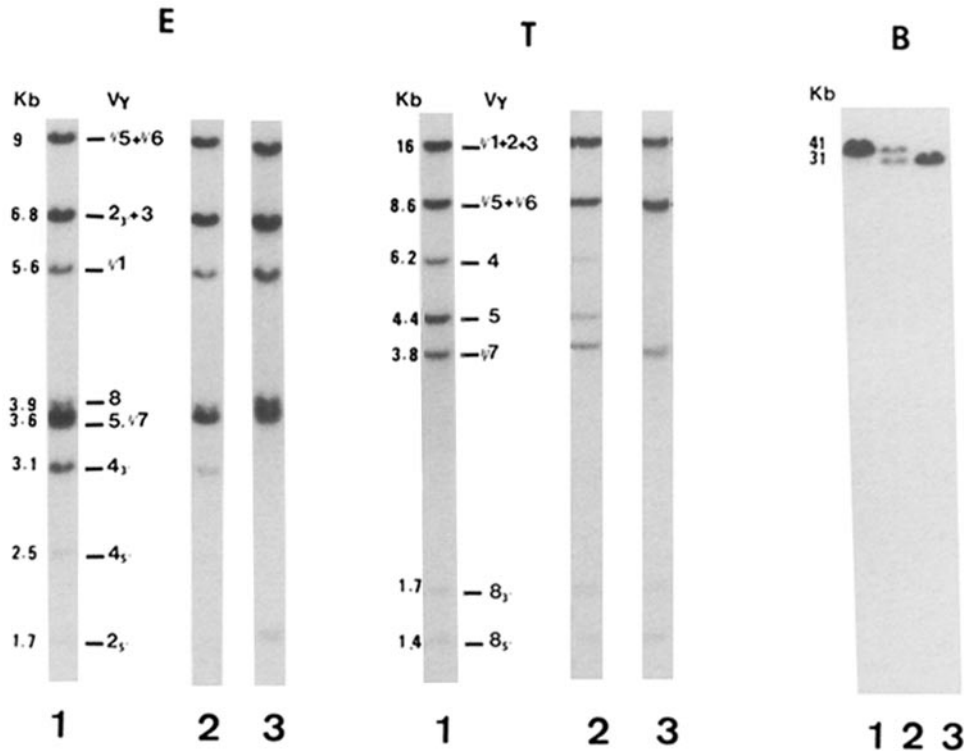
FIGURE 1.   Repertoire polymorphism involving the $V_\gamma 4$ and $V_\gamma 5$ segments. DNA from EBV-transformed cell lines were digested with Eco RI (E), Taq I (T), and Bam HI (B) and hybridized with the $V_\gamma I$ probe. Data obtained from homozygous donors with the large (*1*) and the short (i.e., lacking $V_\gamma 4$ and $V_\gamma 5$) (*3*) haplotypes and from one heterozygous (*2*) are shown. The assignment of the Eco RI bands to given V_γ segments was made according to Forster et al. (13). The assignment of Taq I bands resulted from the study of V_γ deletions in a panel of well-characterized V_γ rearrangements (data not shown).

To demonstrate that the variability in the size of the $V_\gamma I$ region corresponded to a genetic polymorphism, a search for heterozygous subjects was performed and the segregation of $V_\gamma I$ haplotypes was analyzed. Careful analysis of Eco RI and Taq I digests allowed the detection of 26 donors in which the intensity of the DNA fragments corresponding to $V_\gamma 4$ and $V_\gamma 5$ is decreased, which suggest that these donors were heterozygotes. Consistent results were obtained in all cases by two independent analyses. In addition, Bam HI digests from two of these DNAs were also analyzed. As expected from heterozygotes, two Bam HI fragments of 31 and 41 kb, respectively, were observed (Fig. 1 *B*, lane *2*). The segregation of short and normal sized haplotypes was analyzed on 10 informative families. A representative experiment is shown in Fig. 2. In all families, a Mendelian inheritance was observed. These data support the view that a repertoire polymorphism corresponding to the lack of the two $V_\gamma 4$ and $V_\gamma 5$ gene segments exists at the $V_\gamma I$ locus. The frequency of this variant $V_\gamma I$ haplotype is 16%.

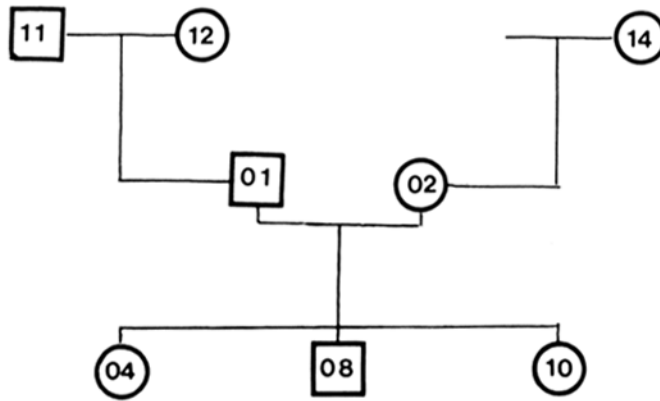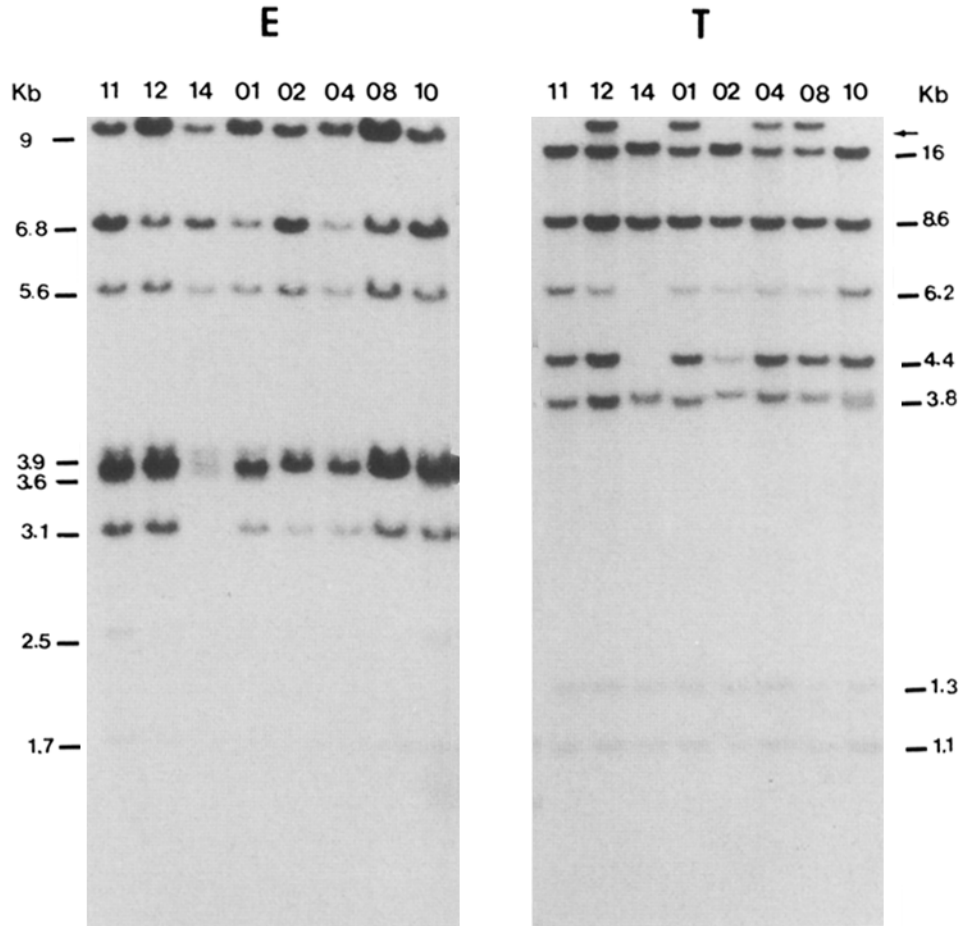*Molecular Cloning of the Polymorphic $V_\gamma 4$-$V_\gamma 5$–containing Region.*   In previous works

FIGURE 2.   Segregation of two polymorphisms in one family. Homozygous donor for the lack of Vγ4-Vγ5: *14*, heterozygous for the same polymorphism: *2*, RFLP involving the Eco RI and Taq I sites 5' to Vγ4. Heterozygous donors: *1*, *4*, *8*, *12*. There is no polymorphism for donors 10 and 11.

(13, 15) the $V_\gamma 5$ gene segment has been mapped to a region between the $V_\gamma 4$ and $V_\gamma 5$ segments, but the precise localization was not defined. Moreover, neither germline $V_\gamma 4$ nor $V_\gamma 5$ genes had been described. It was thus important to clone this region in order to better understand the repertoire polymorphism involving the $V_\gamma 4$ and $V_\gamma 5$ segments. This was done by screening the genomic libraries B16 and DS6 with the $V_\gamma I$ and J_γ probes, respectively. Four overlapping phage clones encompassing 25 kb were obtained and mapped by restriction enzyme digestion. Comparison with previously published maps (12) demonstrated that these clones would cover a DNA region extending from the $V_\gamma 3$ to the $\psi V_\gamma 6$ segment (Fig. 3). A Kpn I site was observed 4.2 kb 3' to $V_\gamma 4$ and 4.5 kb 5' to $\psi V_\gamma 5$. As a Kpn I site is present inside all previously described $V_\gamma I$ segments (12), we hypothesized that the $V_\gamma 5$ segment was localized at this site. A 3.7-kb Eco RI fragment from clone B27 that spanned this Kpn I site and the rearranged $V_\gamma 5$ from clone 601 were subcloned and sequenced. The DNA sequence of the hypothesized germline $V_\gamma 5$ gene (Fig. 4) showed complete identity to the corresponding regions of the rearranged genomic $V_\gamma 5$ segment from clone 601 as well as to a $V_\gamma 5$ cDNA previously published (22). The heptamer/nonamer recognition sequences are similar to that observed 3' to the other $V_\gamma I$ rearranging segments (12). We have also subcloned the two Kpn I containing DNA fragments located 5' and 3' to the $V_\gamma 5$ gene, which could correspond to $V_\gamma 4$ and $\psi V_\gamma 5$ segments. DNA sequencing of these segments showed that they were indeed the expected gene segments. The germline $V_\gamma 4$ gene is identical to the previously described rearranged $V_\gamma 4$ (12) in all corresponding regions and possesses the typical recombination sequences at the 3' end (Fig. 4). On the other hand, the partial sequence (288 bases from ATG initiation codon to the Kpn I site) of the $V_\gamma I$ segment located downstream to $V_\gamma 5$ bore the same DNA sequence as that of the published 5' part of the $\psi V_\gamma 5$ segment apart from a difference of one nucleotide (position 119; C instead of A). This may be due to genetic polymorphism or technical error. Thus, the physical linkage between the $V_\gamma 5$ and other $V_\gamma I$ segments has been definitively established. As shown in Fig. 3, the distance between the Eco RI sites 3' to the $V_\gamma 3$ and 3' to the V_γ 5' segment is 10 kb, corresponding perfectly to the size of the polymorphic $V_\gamma 4$-$V_\gamma 5$ region estimated by the Southern technique.
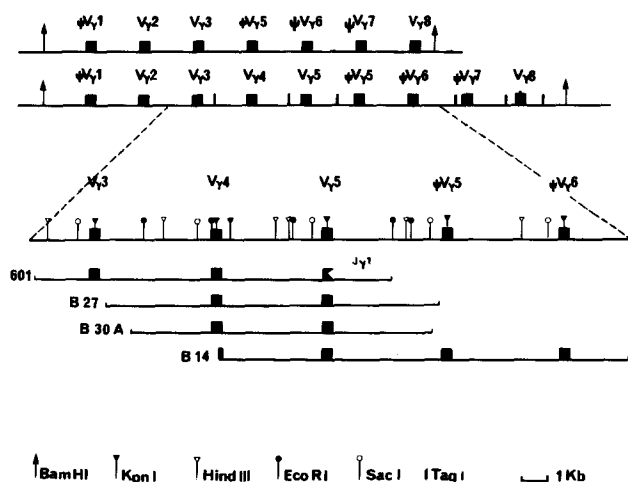

FIGURE 3. Partial restriction map of the $V_\gamma I$ family. B27, B30A, and B14 clones were obtained from the B16 genomic library (15). The 601 clone contains a $V_\gamma 5$ segment rearranged to the $J_\gamma 1$ segment and was cloned from the DS6 library.

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Vγ4  ATGCAGTGGGCCCTAGCGGTGCTTCTAGCTTTCCTGTCTCCTGGTGAGTGCGCTGCCTAC
Vγ2  .............................................................

Vγ4  AGAGAGGATCATGGGTTTTGTTTTCTTTATTTTCTTCTTTTGCAAGGATTGCCATACTAA
Vγ2  .........C.........            ..................G.A.........

Vγ4  GGAATTCCTCATTATATTTTGTGTTGTTCCCATTGCAGCCAGTCAGAAATCTTCCAACTT
Vγ2  ...............................T............................

Vγ4  GGAAGGGAGAACGAAGTCAGTCATCAGGCAGACTGGGTCATCTGCTGAAATCACTTGTGA
Vγ2  ............................................................

Vγ4  TCTTGCTGAAGGAAGTACCGGCTACATCCACTGGTACCTACACCAGGAGGGGAAGGCCCC
Vγ2  ..............A.............................................

Vγ4  ACAGCGTCTTCTGTACTATGACTCCTACACCTCCAGCGTTGTGTTGGAATCAGGAATCAG
Vγ2  ...........A...............A.....AG..................G...'.

Vγ4  CCCAGGGAAGTATGATACTTACGGAAGCACAAGGAAGAACTTGAGAATGATACTGCGAAA
Vγ2  T..........T.........C..............C.........T.............

Vγ4  TCTTATTGAAAATGACTCTGGAGTCTATTACTGTGCCACCTGGGATGGGCACAGTGATTC
Vγ2  ....A..................G...........................C.........

Vγ4  AGATCCGCCCTACACCACACTGAAAACC
Vγ2  .........................T.
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Vγ5  ATGCGGTGGGCCCTACTGGTGCTTCTAGCTTTCCTGTCTCCTGGTGAGTACGCTGCCTAC
Vγ3  ...............GC...........................................

Vγ5  AGAGAGGCTCACAGGTTGGGTTTTGTTTTGTTTACTTCTTTTGAAAGGGGTGCGATACAA
Vγ3  ...................................T.......          .C......

Vγ5  AGGAATACCTCATTAAATTTTGTGTTGTTCCCATTGCAGCCAGTCAGAAATCTTCCAACT
Vγ3  ..............GT............................................

Vγ5  TGGAAGGGGGAACGAAGTCAGTCACGAGGCCGACTAGGTCATCTGCTGAAATCACTTGTG
Vγ3  .........A..............C....A....G....................C.

Vγ5  ACCTTACTGTAATAAATGCCTTCTACATCCACTGGTACCTACACCAGGAGGGGAAGGCCC
Vγ3  .T.........C.....A..........................................

Vγ5  CACAGCGTCTTCTGTACTATGACGTCTCCAACTCAAAGGATGTGTTGGAATCAGGACTCA
Vγ3  ........................C.G....G............................

Vγ5  GTCCAGGAAAGTATTATACTCATACACCCAGGAGGTGGAGCTGGATATTGATACTACGAA
Vγ3  .................................................G...G.A..

Vγ5  ATCTAATTGAAAATGATTCTGGGGTCTATTACTGTGCCACCTGGGACAGGCACAGTGATT
Vγ3  ............................................................

Vγ5  CAGACCTGTCCTACACCACACTGAAAATC
Vγ3  .............................
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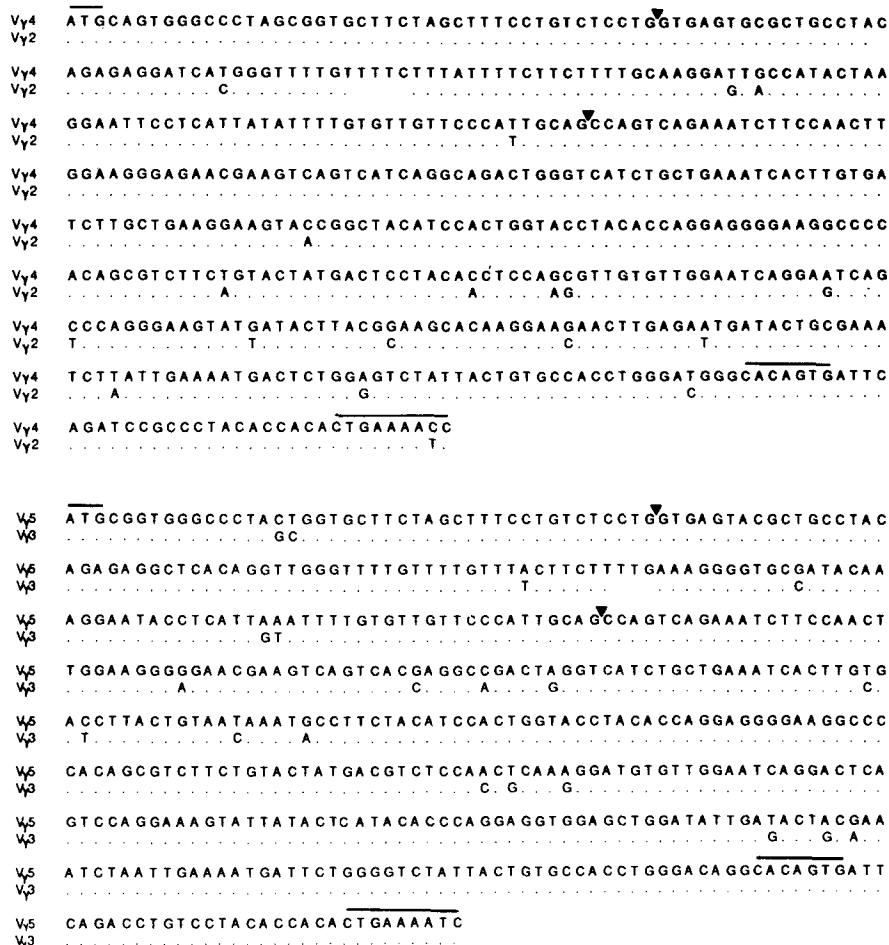
FIGURE 4.   Comparison of the DNA sequence of the germline Vγ4 (this study) with that of the Vγ2 (12) segment (top of the figure) and of the germline Vγ5 (this study) with that of Vγ3 (12) (bottom). The ATG initiation codon and the heptamer-nonamer recognition sequences are overlined. The splicing sites are indicated by arrow. These sites within Vγ2, Vγ3, and Vγ4 segments are described in reference 12. Those in Vγ5 were deduced from the comparison of the DNA sequence of genomic Vγ5 (this study) with that of a cDNA previously published (22).

Examination of the organization of Vγ2-Vγ3 and Vγ4-Vγ5 demonstrated some resemblance in restriction maps (reference 12 and Fig. 3). There is an Eco RI site in introns of Vγ2 and Vγ4 segments and two closely located Hind III sites are present 5' to Vγ3 and Vγ5 segments. This prompted us to compare the nucleotide and deduced amino acid sequences of these gene segments. As shown in Fig. 4, a striking homology of nucleotide sequences is present between Vγ2 and Vγ4 (95%) and between Vγ3 and Vγ5 (95%). This homology is higher than those between Vγ2 and Vγ3 (88%), and between Vγ4 and Vγ5 (86%). The differences in nucleotide sequence occurs mainly in the intron and three hypervariable regions. Comparison of deduced amino acid sequences shows the same result (Fig. 5), with significantly

higher homologies between $V_\gamma 2$ and $V_\gamma 4$ (91%) and between $V_\gamma 3$ and $V_\gamma 5$ (90%) than that observed between $V_\gamma 2$ and $V_\gamma 3$ and between $V_\gamma 4$ and $V_\gamma 5$ (76% for both comparisons). These data as discussed below should help us to explore the possible mechanism underlying the repertoire polymorphism concerning the $V_\gamma 4$-$V_\gamma 5$ region and the $V_\gamma 2$-$V_\gamma 3$ segments (see below). The organization of the normal sized $V_\gamma I$ subgroup and the short-sized haplotype that lacks $V_\gamma 4$ and $V_\gamma 5$ are shown in Fig. 3.

Four Other Polymorphisms Involving the $V_\gamma I$ Subgroup. Two other polymorphisms consistent with a repertoire polymorphism were each found in one individual. In one DNA sample, additional 9-kb Taq I fragments were noted, while intensity of the $V_\gamma 2$-$V_\gamma 3$-containing fragment was decreased in both Taq I and Eco RI digests (Fig. 6, panel *1*). The corresponding $V_\gamma I$ allele appeared to be reduced by ~10 kb in size on Bam HI digestion (not shown), consistent with a $V_\gamma 2$-$V_\gamma 3$ deletion on one chromosome. In another DNA sample, additional strong hybridizing 6.1-kb Eco RI and 10.5-kb Taq I fragments were observed (Fig. 6, panel *2*). Intensity of the $V_\gamma 4$-containing fragments was decreased in both digests, but other bands demonstrated the usual pattern. This could correspond to an insertion of a V_γ^- containing DNA fragment in a site 5' to $V_\gamma 4$ and 3' to $V_\gamma 3$. Segregation of these two polymorphisms was demonstrated in informative families (not shown).

Frequent restriction fragment length polymorphisms (RFLP) were documented in addition to the repertoire polymorphisms. An additional 22-kb Taq I fragment was found in DNA samples from 10 nonrelated individuals (Fig. 2 *T*, lanes *2*, *4*, *8*, and *12*). The intensity of the usual $V_\gamma 1$-2-3- (16 kb) and $V_\gamma 4$- (6.2 kb) containing bands was decreased in these samples. This pattern could be due to the lack of the Taq I site located 5' to $V_\gamma 4$, leading to a 22-kb band that included the $V_\gamma 1$, $V_\gamma 2$, $V_\gamma 3$, and $V_\gamma 4$ segments. Interestingly, this was associated with an Eco RI RFLP in all these individuals. This resulted in a new 9.3-kb band that was only clearly distinguishable from the usual $\psi V_\gamma 5$-$\psi V_\gamma 6$ fragments in long-run electrophoresis ex-

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VY2    QKSSNLEGRTKSVIRQTGSS
VY3    QKSSNLEGRTKSVIRQTGSS
VY4    QKSSNLEGRTKSVIRQTGSS
VY5    QKSSNLEGGTKSVIRETRSS

VY2    AEITCDLAEGSNGYIHWYLH
VY3    AEITCDLTVINIEYIHWYLH
VY4    AEITCDLAEGSTGYIHWYLH
VY5    AEITCDLTVINAEYIHWYLH

VY2    QEGKAPQRLQYYDSYNSKVV
VY3    QEGKAPQRLLYYDVSTARDV
VY4    QEGKAPQRLLYYDSYTSSVV
VY5    QEGKAPQRLLYYDVSNSKDV

VY2    LESGVSPGKYYTYASTRNNL
VY3    LESGLSPGKYYTHTPRRWSW
VY4    LESGISPGKYDTYGSTRKNL
VY5    LESGLSPGKYYTHTPRRWSW

VY2    RLILRNLIENDSGVYYCATW
VY3    ILRLONLIENDSGVYYCATW
VY4    RMILRNLIENDSGVYYCATW
VY5    ILILRNLIENDSGVYYCATW

VY2    DG
VY3    DR
VY4    DG
VY5    DG
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FIGURE 5. Amino acid sequence homology between $V_\gamma 2$, $V_\gamma 3$, $V_\gamma 4$, and $V_\gamma 5$ gene segments. The $V_\gamma 2$ segment is used as reference. The differences are boxed.
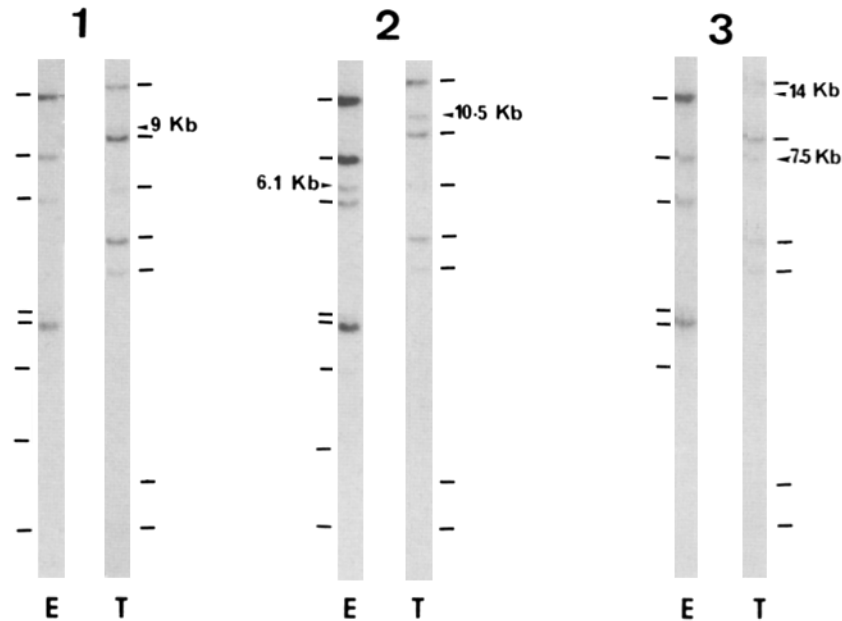
FIGURE 6.   Three polymorphisms, each observed in only one DNA sample. *1*, deletion of V$_\gamma$2-V$_\gamma$3; *2*, insertion of a DNA sequence 5′ to V$_\gamma$4; *3*, association of a lack of Taq I and Eco RI sites 5′ to V$_\gamma$4 with a new Taq I site inside the V$_\gamma$1-V$_\gamma$2-V$_\gamma$3-V$_\gamma$4 region; lack of V$_\gamma$4 and V$_\gamma$5 in the other haplotype (see text). DNA were digested with Eco RI (E) and Taq I (T) and hybridized with the V$_\gamma$I probe. (▶) Polymorphic bands; (–) nonpolymorphic bands.

periments. Appearance of this new band was associated with decreased intensity of the 6.8-kb V$_\gamma$2-V$_\gamma$3 Eco RI fragment (Fig. 2 *E*, lanes *1, 4, 8*, and *12*). This pattern is consistent with the lack of the Eco RI site located near the Taq I site 5′ to V$_\gamma$4. The segregation of this polymorphism was studied in six families, showing a Mendelian inheritance (Fig. 2). The haplotypic frequence of the combined lack of these Taq I and Eco RI sites is 6.9% in the random population. Finally, a complex pattern was observed in a last sample (Fig. 6, panel *3*). In addition to the lack of the Taq I site 5′ to V$_\gamma$4, a new Taq I site was present inside the ψV$_\gamma$1-V$_\gamma$2-V$_\gamma$3-V$_\gamma$4 fragment generating two fragments of 7.6 and 13.5 kb. The Eco RI 5′ to V$_\gamma$4 was also lacking in the same chromosome. On the other haplotype, there was a lack of V$_\gamma$4 and V$_\gamma$5.

Discussion

In this paper, we have shown that two types of polymorphisms frequently occur in the first subgroup of the T cell γ chain gene variable segments. First, we have documented two RFLP by Southern and genetic analyses. More interestingly, we have also described three repertoire polymorphisms, the most frequent of which involved the V$_\gamma$4 and V$_\gamma$5 segments located in a region that has not been fully defined to date. We have cloned this region and sequenced these segments in germline configuration.

Previous studies have documented RFLP occurring in TCR-β and -α V genes

(24–26). In most cases, it was not possible to precisely define the localization of the restriction sites involved in these polymorphisms. The limited repertoire of T cell γ genes and the almost complete characterization of their organization allow easy identification of the gene segments involved by Southern blotting and thus facilitate the analysis of restriction patterns. Recently, RFLP involving a Hind III site located 5' to $V_\gamma 9$ segment ($V_\gamma III$ subgroup) was described by Forster et al. (13), but the frequency of this remains to be defined. In parallel with the present study, we have analyzed with $V_\gamma II$, $V_\gamma III$, and $V_\gamma IV$ probes 72 DNA samples from unrelated individuals. These samples were digested with Eco RI, Hind III, Taq I, and Sac I restriction enzymes. No polymorphism was detected, except in Taq I digests hybridized with the $V_\gamma IV$ probe. This polymorphism consists of two allelic fragments of 3.85 and 4.1 kb whose frequencies are 43.75% and 56.25%, respectively (our unpublished data).

Murine V segment repertoire polymorphisms have been documented relatively frequently. SJL mice lack approximately half of the normal V_β segment repertoire (27), while deletion of one or two members of several V_α subfamilies exists in some strains (26). In contrast, there are only rare examples of polymorphic variation in the number of human TCR V_β segments. In an analysis of 100 DNA samples from unrelated individuals, Concannon et al. (24) have documented one case of homozygote deletion of one $V_\beta 6$ member. Our study shows that the number of $V_\gamma I$ segments may vary from one individual to another, with the most frequent polymorphism consisting of the presence or absence of both $V_\gamma 4$ and $V_\gamma 5$ segments. The lack of $V_\gamma 4$ and $V_\gamma 5$ is observed in 16% of haplotypes. In an attempt to explore possible mechanisms involved in this polymorphism, we have cloned a DNA fragment spanning this polymorphic DNA region, determined its restriction map, and sequenced relevant $V_\gamma I$ segments. From this analysis, it appears that the restriction map of the $V_\gamma 2$-$V_\gamma 3$ region is quite homologous to that of the $V_\gamma 4$-$V_\gamma 5$ region. More importantly, the DNA and deduced amino acid sequences of $V_\gamma 4$ and $V_\gamma 5$ are very similar to that of $V_\gamma 2$ and $V_\gamma 3$, respectively (Fig. 5). The formation of the $V_\gamma 4$-$V_\gamma 5$–containing haplotype is most likely to represent a recent duplication involving the $V_\gamma 2$-$V_\gamma 3$ region. We can not however exclude that deletion of $V_\gamma 4$ and $V_\gamma 5$ could have generated the shorter $V_\gamma I$ haplotype. Interestingly, in one haplotype, the reverse situation was observed: deletion of $V_\gamma 2$-$V_\gamma 3$ segments with conservation of the $V_\gamma 4$-$V_\gamma 5$ region. Taken together with the strong possibility of a gene duplication event between the $V_\gamma 2$-$V_\gamma 3$ and $V_\gamma 4$-$V_\gamma 5$ segments, one can hypothesize that there may be some special sequences flanking the $V_\gamma 2$-$V_\gamma 3$ region that may favor genetic recombinations.

The biological implication of the repertoire polymorphisms described in this paper is unknown. The homology at the protein level between $V_\gamma 2$-$V_\gamma 3$ and $V_\gamma 4$-$V_\gamma 5$ is considerable. This implies that in homozygotes for the short $V_\gamma I$ haplotype, the lack of $V_\gamma 4$ and $V_\gamma 5$ could be compensated to some extent by the $V_\gamma 2$-$V_\gamma 3$ products. However, it is worth noting that there is some difference in the amino acid sequences coded by these two groups of $V_\gamma I$ genes (8.7–9.6%). The replacement of only one amino acid at a critical region could considerably modify the binding affinity or specificity as previously demonstrated by the structure-function study of Ig or polymorphic MHC products (28, 29). As γδ expressing cells are probably involved in some important immunological functions (30), it is possible that this polymorphism

may induce some alteration of immune response. Associations between this repertoire polymorphism and some immune disturbances are now under study.

Summary

Southern blot analysis using a genomic probe of the human TCR-γ chain first variable gene subgroup (V$_\gamma$I) was performed on DNA samples from both parents of 36 healthy Caucasian families. Two types of polymorphisms were found in these 72 unrelated DNA samples: three repertoire polymorphisms and two restriction fragment length polymorphisms (RFLP). In all cases, Mendelian inheritance of these polymorphisms was demonstrated. The most frequent repertoire polymorphism consists in the lack of the V$_\gamma$4 and V$_\gamma$5 segments. In 16% of chromosomes, the Eco RI and Taq I restriction fragments corresponding to V$_\gamma$4 and V$_\gamma$5 were lacking, with no additional bands. In these cases, a decrease of 10 kb was observed in the Bam HI fragment containing all V$_\gamma$I segments as compared with samples containing V$_\gamma$4-V$_\gamma$5 segments. To better understand this polymorphism, which takes place in a previously incompletely defined region, the central part of the V$_\gamma$I region, including the polymorphic V$_\gamma$4-V$_\gamma$5 segments, was cloned. This allowed us to localize precisely the V$_\gamma$5 segment and thus complete the description of the V$_\gamma$I region. A striking homology of DNA and deduced amino acid sequences is present between V$_\gamma$2 and V$_\gamma$4 and between V$_\gamma$3 and V$_\gamma$5, much higher than that observed between V$_\gamma$2 and V$_\gamma$3 and between V$_\gamma$4 and V$_\gamma$5. The differences in nucleotide sequence occur mainly in the intron and three hypervariable regions. These results strongly suggest a gene duplication relationship between the segments V$_\gamma$2-V$_\gamma$3 and the segments V$_\gamma$4-V$_\gamma$5. The most frequent RFLP documented in this study is due to the combined absence of the Eco RI and the Taq I sites located in the noncoding region between V$_\gamma$3 and V$_\gamma$4. The haplotypic frequence of this RFLP is 6.9% of the general population. As the γ/δ receptor may play an important role in immunological response, the biological relevance of the high degree of polymorphism occurring in the V$_\gamma$I region, as well as its possible association with some immune disturbances, should be further explored.

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