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New subgroups in the human T cell rearranging V_γ gene locus

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Two new V_γ genes in humans are described from rearrangement in T cell lines, which constitute single members of new V gene subgroups of the T-cell rearranging γ (TRG γ) locus. These two genes (herein designated as belonging to V_γIII and V_γIV subgroups) are located between V_γI/V_γII subgroups and the constant (C) γ genes. The existence of these new genes brings the number of different, potentially useable, human TRG V_γ genes to eight (excluding at least five pseudo V_γ genes) and the number of distinct subgroups to four. Polymorphism in the sequence of the V_γII subgroup gene is also described and rearranged fragment sizes which make possible an unequivocal assignment of a V_γ rearrangement are given. These results extend previous conclusions of the inherited diversity of the human TRG V_γ locus.

Key words: T cell rearranging γ gene/V_γ subgroup/T cell receptor

Introduction

The T cell rearranging gene γ (TRG γ) was first recognized in mouse cells (Saito *et al.*, 1984). The TRG γ locus, like other studied rearranging genes, has variable (V) region genes, joining (J) and constant (C) region segments (Hayday *et al.*, 1985) which join during the early stage of T cell differentiation (Raulet *et al.*, 1985).

There are significant differences between the TRG γ locus in man and mouse. In man, there have been identified so far two C_γ genes (Lefranc and Rabbitts, 1985; Dialynas *et al.*, 1986; Lefranc *et al.*, 1986a) which are separated by 16 kb (Lefranc and Rabbitts, 1985; Lefranc *et al.*, 1986a) and a set of V_γ segments which are shared by the two C_γ genes (Lefranc *et al.*, 1986b,c). The situation in mouse is different since the four C_γ genes (Kranz *et al.*, 1985; Iwamoto *et al.*, 1986) each have associated V_γ and J_γ segments. This comparative anatomy of the human and mouse TRG γ loci draws a strong parallel with the immunoglobulin lambda light chain gene loci (Lefranc *et al.*, 1986a).

The function of the TRG γ gene product is presumably to form a surface receptor molecule on T cells, analogous to the TCR α/β heterodimer but the function of this TRG receptor and the T-cell type bearing this receptor is only just beginning to be clarified. A subset of T cells, expressing CD3 (T3) but not CD4 (T4) or CD8 (T8) has recently been identified which express γ chains (Brenner *et al.*, 1986; Bank *et al.*, 1986; Weiss *et al.*, 1986) possibly in association with a new chain, designated δ. γ expression has also been demonstrated on the surface of NK cells (Moingeon *et al.*, 1986). These studies support the view that the TRG γ locus codes for a diverse set of receptor proteins which, in turn, can recognize a diverse set of antigens. Sequence studies on a number of the human V_γ genes has allowed the assignment of two distinct subgroups V_γI and V_γII (Lefranc *et al.*, 1986b,c) but rearrangement patterns of TRG γ in the DNA of T cell leukaemias suggested the presence of, as yet, undetected V_γ subgroups (Lefranc and Rabbitts, 1985; Murre *et al.*, 1985; Quertermous *et al.*, 1986). We now describe a new V_γ subgroup (V_γIII) by the isolation of a gene from a γ rearrangement present in the cell line RPMI 8402 and identify a fourth V_γ subgroup (V_γIV) by its rearrangement in the JM cell line. The localization of these new genes and a polymorphism of the V_γII subgroup is discussed.

Results

A new TRG γ subgroup gene, V_γIII

The set of V_γ genes previously described (Lefranc *et al.*, 1986b) as belonging to the V_γI and V_γII subgroups could be assigned to the major rearrangements observed in human foetal thymus samples but a further, very faint rearranged fragment could be detected in some samples. This, together with an analysis of a large number of T-cell lines (Lefranc and Rabbits, 1985; Murre *et al.*, 1985), suggested that there existed at least one other V_γ family, in addition to V_γI and V_γII. Examination of the DNA of RPMI 8402 revealed two rearranged TRG γ alleles, one of which could be assigned to the V_γI family (unpublished, and see below) and the other which did not fit with any hitherto isolated genes (unpublished).

We have, therefore, isolated the rearranged V_γ genes from a λ phage library made from RPMI 8402 DNA by screening with a J_γ probe [pH60; Lefranc and Rabbitts (1985) and Materials and methods]. Two groups of clones were analysed representing the two rearranged alleles and the restriction maps appears in Figure 1. Both chromosomes are rearranged to the $C_\gamma 2$ gene and both have joins to the $J_\gamma 2$ segment previously identified (Lefranc *et al.*, 1986c). One allele (Figure 1A) had an unassigned V_γ gene joined to $J_\gamma 2$ (see below) and the homologous allele (Figure 1B) has the $V_\gamma 4$ gene (subgroup V_γI) joined to $J_\gamma 2$ as judged by the restriction map of λR11 and λR9 and comparison to unrearranged C_γ, J_γ and V_γ regions. Since the various segments in the latter have been previously analysed (Lefranc *et al.*, 1986b), we have carried out no further analysis on these rearranged λ phage clones.

A restriction map of the λR12 and λR7 clones which carried the unidentified, rearranged DNA segments is shown in Figure 1A. Comparison of this map with the map of the unrearranged C_γ locus (Lefranc *et al.*, 1986a) showed that a rearrangement had occurred at $J_\gamma 2$. This rearrangement was examined by DNA sequencing and revealed that a V-region gene had joined, non-productively, to $J_\gamma 2$ (Figure 2A). The in-coming DNA segment

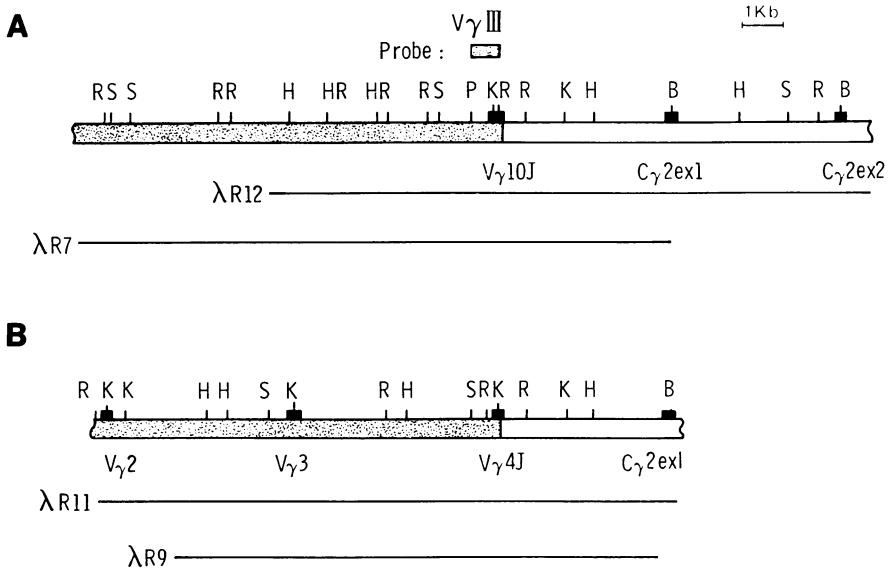

Fig. 1. Restriction enzyme maps of rearranged V_γ gene in RPMI 8402. λ phage clones were isolated from a library of RPMI 8402 DNA (in λ 2001) using the $J_\gamma1$ probe pH60. Two representative clones corresponding to each allele are shown together with the deduced restriction map. Black boxed areas represent V_γ genes, labelled accordingly. (**A**) Rearranged allele with $V_\gamma10$-$J_\gamma2$; (**B**) rearranged allele with $V_\gamma4$-$J_\gamma2$. R, *Eco*RI; S, *Sac*I, H, *Hin*dIII; P, *Pst*I (only relevant sites shown); K, *Kpn*I; B, *Bam*HI.

could be readily identified as a V_γ gene by certain highly conserved codons present; e.g. the conserved cysteine codons (codons 18 and 93) which are probably responsible for intrachain disulphide bonding of the V region, the tryptophan–tyrosine dipeptide (associated with the conserved *Kpn*I restriction enzyme site) at codons 32 and 33 and the codons for tyrosine–tyrosine–cysteine–alanine tetrapeptide around the C terminus of the V region. Although clearly a V-region sequence, the V gene rearranged in λR12 and λR7 has very limited homology to the other known V_γ genes and, therefore, this gene ($V_\gamma10$) belongs to a third, distinct subgroup which we term V_γIII. The lack of homology of this V_γIII subgroup gene to, for instance, $V_\gamma9$ (V_γII subgroup) is illustrated in Figure 2B where an alignment of the derived V-region protein sequence is compared. The most homologous region is in the third framework region (from residues 83 to 94) and the overall homology (ignoring a simple space introduced into $V_\gamma9$ to maximize homology) is 34%. This, and the previously reported homologies of ~30% between putative proteins of V_γI and V_γII (Lefranc *et al.*, 1986b) show that subgroups are markedly different from each other and signify the ability of a putative receptor, involving the γ chain, to recognize diverse antigens.

Mapping of the V_γIII subgroup

By studying the organization and rearrangement of the various V_γ genes, it was shown that the two identified C_γ loci could rearrange with any of the V_γ genes and that the V_γII gene ($V_\gamma9$) was located between the V_γI cluster and the C_γ genes on the chromosome (Lefranc *et al.*, 1986b). The chromosomal position of the V_γIII subgroup was determined by deletion mapping using genomic DNA from a cell line (SUP-TI) which has both TRG γ alleles rearranged to V_γI subgroup genes [$V_\gamma3$ and $V_\gamma4$ respectively (Lefranc *et al.*, 1986b)].

The V_γIII subgroup probe is illustrated in Figure 1A and the hybridization of this probe to genomic DNA of various sources is shown in Figure 3. In an experiment, performed under low stringency, we detected one major component of hybridization (~1.7 kb in *Eco*RI digests) in DNA from a non-lymphoid cell line (Colo 320) indicating that there is a single V_γIII gene in the human genome (i.e. the $V_\gamma10$ gene); no cross hybridization was observed between V_γIII and either V_γI or V_γII genes. DNA from RPM1 8402 and from K1010 cells exhibit the hybridizing V_γ gene of the same mobility showing that these DNAs both carry the $V_\gamma10$ gene [using *Eco*RI digests, the $V_\gamma10$ gene does not appear rearranged since this enzyme cleaves within the V gene (see Figure 2A)]. However, no hybridization of the SUP-TI DNA was observed with the V_γIII probe showing that deletion of this gene has occurred on both chromosomes in this cell line. Since this cell has rearrangements involving $C_\gamma2$ with $V_\gamma3$ on one chromosome and with $V_\gamma4$ on the other the deletion of $V_\gamma10$ indicates that this gene is between $V_\gamma4$ and the two C_γ genes. Given the organization of the human V_γ locus, the most likely location for $V_\gamma10$ is between $V_\gamma9$ and $C_\gamma1$.

Polymorphism of the $V_\gamma9$ gene

Although there is no evidence for somatic mutation occurring in the V_γ genes (Hayday *et al.*, 1985; Lefranc *et al.*, 1986b), N-region diversity does contribute significantly to somatic variation of these genes (Hayday *et al.*, 1985; Lefranc *et al.*, 1986b; Quertermous *et al.*, 1986). The inherited sequence variation of the V_γ genes in man may also be increased by genetic polymorphism within the human population as a whole. This possibility was investigated in the $V_\gamma9$ gene because we observed an RFLP within the region flanking $V_\gamma9$ in λ clones isolated from different cellular DNAs (Figure 4A). When the restriction map of λK20 (isolated from K1010 DNA) was compared with those of λA6 (isolated from AT5B1 DNA) and of λSHV7 (isolated from a B-cell lymphoblastoid cell line), we noted an RFLP of a *Hin*dIII fragment adjacent to the 5′ end of $V_\gamma9$; this fragment is 3.1 kb in λ K20 and 2.75 kb in λA6 and λSHV7. The nucleotide sequence comparison of an ~450-bp region obtained from both λ K20 and λ A6 $V_\gamma9$ genes, shows that three nucleotide changes have occurred (Figure 4B). Two of these changes occur within the coding region and one of these results in a lysine-to-threonine

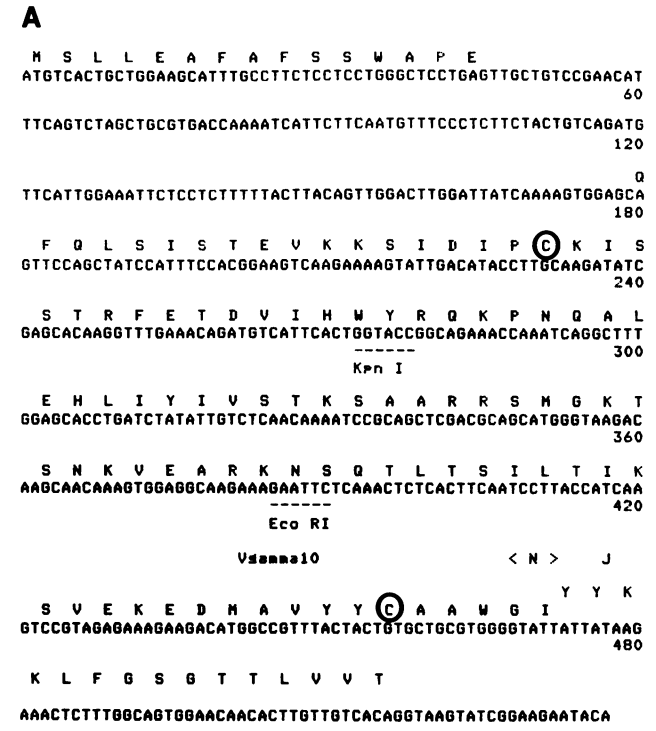

Fig. 2. Sequence of the $V_\gamma10$ rearrangement. (**A**) Nucleotide sequence and deduced amino acid sequence of $V_\gamma10$ non-productively joined to $J_\gamma2$. Cysteines probably involved in intra-chain disulphide bonds are circled. Position of relevant *Kpn*I and *Eco*RI sites are shown. (**B**) Alignment of $V_\gamma10$-derived protein sequence with $V_\gamma9$. Stars indicate identity of the amino acid and the intra-chain cysteines are specified by angled lines.

replacement. Although the effect of such a replacement on the folding of the V_γ protein is not clear, such a replacement is likely to be significant, and thus the V-region variability between polymorphic individuals would necessarily be increased by this change.

Localization of $V_\gamma11$ representing a V_γIV subgroup

JM cells (equivalent rearrangements to Jurkatt) have two rearranged TRG_γ alleles. The DNA cut with *Bam*HI, *Eco*RI or *Hin*dIII and hybridized with J_γ probes, shows two rearrangements one of which can be identified as a rearranged $V_\gamma8$ gene (4.2-kb *Eco*RI, Figure 5A and Table IIB). The other rearranged band (9.5-kb *Eco*RI, Figure 5A) corresponds to a new gene, $V_\gamma11$, which must belong to a new subgroup V_γIV since there is no hybridization of a V_γI probe to such a rearranged band (the only rearranged band detected is the 4.2-kb $V_\gamma8$) and since V_γII and III subgroups only contain one member each. Furthermore,

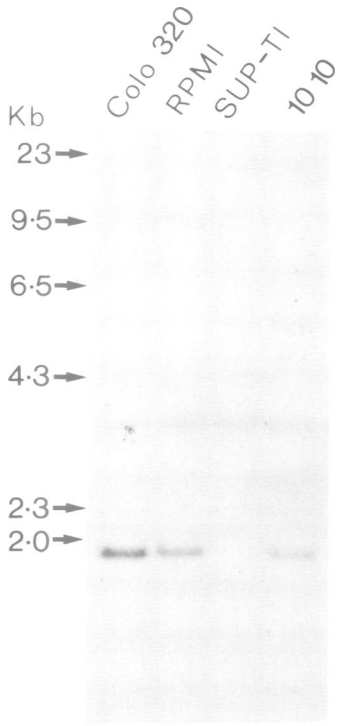

Fig. 3. Mapping V_γIII subgroup within the human V_γ locus. Genomic hybridization using the $V_\gamma10$ probe illustrated in Figure 1A. DNA from COLO32OHSR [a colon carcinoma (Alitalo *et al.*, 1983)] RPMI 8402, SUP-T1 [a T-cell lymphoma (Hecht *et al.*, 1984)] and K1010 (Lefranc *et al.*, 1986b) was digested to completion with *Eco*RI, separated on 0.8% agarose and transferred to cellulose nitrate as described (Lefranc *et al.*, 1986b). After hybridization with the V_γIII probe, the filter was washed at 6 × SSC, 0.1% SDS prior to autoradiography. Sizes shown are from λDNA cleaved wtih *Hin*dIII.

Jurkatt cells have been shown to contain a previously unidentified rearranged gene by partial sequence data at the V−J junction (Quertermous *et al.*, 1986).

The location of this $V_\gamma11$ gene was deduced by V_γ gene hybridization to JM DNA. A V_γI subgroup probe (1.1-kb *Sac*I $V_\gamma3$ fragment from λSH4) detects a normal pattern of *Eco*RI bands, including $V_\gamma8$ in the unrearranged configuration (this band is faint and merges with the intense band due to $V_\gamma5$ and $\psi V_\gamma7$) plus the 4.2-kb $V_\gamma8$ rearranged band (Figure 5B). Hybridization of a V_γII probe [K20PR, the *Pst*−*Eco*RI $V_\gamma9$-J_γ fragment from λK20 (Lefranc *et al.*, 1986c)] detects, in addition to the fragments observed with a J_γ probe, the 5.2-kb fragment which represents the unrearranged $V_\gamma9$ gene (data not shown). Similarly, the V_γIII probe shows the unrearranged 1.7-kb *Eco*RI (Figure 5C) band so that $V_\gamma8$, 9 and 10 genes must still be present on the chromosome with rearranged $V_\gamma11$. Therefore, $V_\gamma11$ must be downstream of $V_\gamma10$, assuming no inversion mechanism.

The germline V_γI genes can be assigned to the different bands in Figure 5B based on cloned DNA fragments (Lefranc *et al.*, 1986b). However, to confirm the assignment, we carried out deletion mapping in T cell lines with different rearranged V_γ genes and in so doing, identified a new V_γI subgroup gene herein designated $V_\gamma5$ and located between $V_\gamma4$ and $\psi V_\gamma5$. The *Eco*RI fragment containing $V_\gamma5$ comigrates with $\psi V_\gamma7$ but this doublet is separable into the two components on longer electrophoretic runs (data not shown).

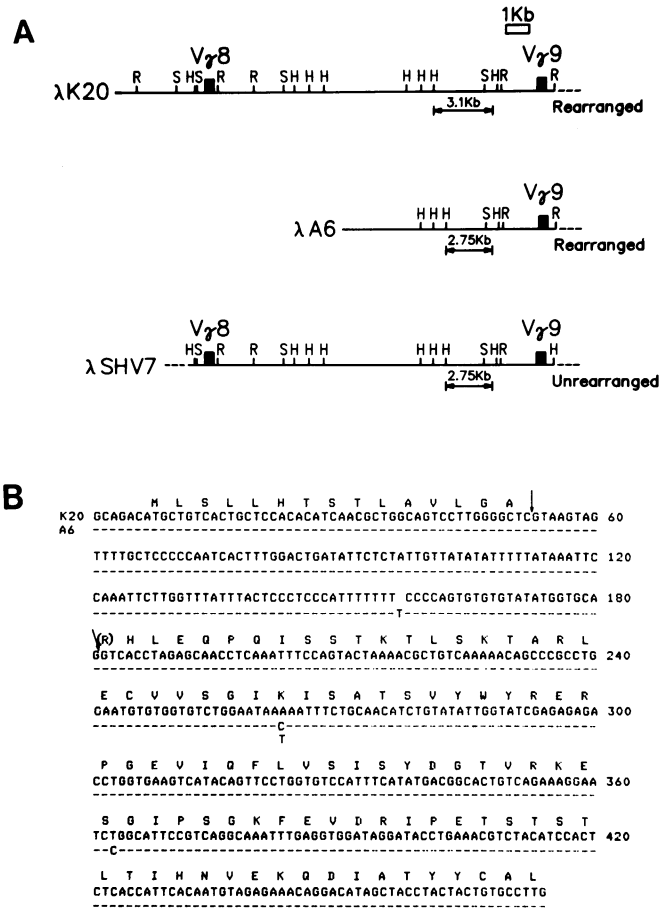

Fig. 4. Genetic polymorphism of the $V_\gamma 9$ gene. (**A**) RFLP in λ clones containing $V_\gamma 9$. λ recombinant phage clones were isolated from either K1010 DNA, AT5B1 or SH DNA containing the $V_\gamma 9$ gene. The restriction map revealed a *Hin*dIII RFLP (indicated by the horizontal arrow) just upstream of the V_γ coding region. R, *Eco*RI; S, *Sac*I, H, *Hin*dIII. (**B**) Comparison of nucleotide sequence of $V_\gamma 9$ genes from λK20 and λA6. Vertical arrows represent putative RNA splice sites.

A pseudo V_γ gene between $V_\gamma 8$ and $V_\gamma 9$ and a possible pseudo V_γ between $V_\gamma 10$ and $V_\gamma 11$

The even genomic spacing of the V_γ genes implies the occurrence of an equal expansion of the V_γ repertoire in man during the evolution of the system; however, this equal spacing of ~5 kb, maintained between the V_γI family genes, was not found between the V_γI family and the V_γII gene distance of 15 kb (Lefranc *et al.*, 1986b). This situation suggested the possibility that there might exist a further V_γ gene segment between $V_\gamma 8$ and $V_\gamma 9$. Furthermore, all of the V_γI family genes possess the conserved tryptophan–tyrosine codons which are associated with a *Kpn*I restriction enzyme site. A similar situation occurs in the $V_\gamma 10$ gene (Figure 2A). Since analysis of the λ A6 and λK20 clones revealed a *Kpn*I site ~6 kb upstream of $V_\gamma 9$, this site might correspond to a new V_γ segment. This was investigated by nucleotide sequencing around this *Kpn*I shown in Figure 6A. In confirmation of this idea that a V_γ segment occurs in this region, the conserved tryptophan–tyrosine and cysteine codons (Figure 6B) were found. However, a translation stop codon (TAG) occurs nine codons upstream of this conserved cysteine codon, thus making this segment a pseudo V_γ segment (here designated $\psi V_\gamma A$).

Mapping by Southern hybridization analysis allowed us (see above) to localize $V_\gamma 11$ downstream of $V_\gamma 10$ (summarized in Figure 7). Hybridization of *Kpn*I-digested JM DNA with the J_γ probe pH60 detected a 6.2-kb fragment instead of the 1.8-kb fragment expected from V_γ genes with internal *Kpn*I sites, indicating that $V_\gamma 11$ (subgroup IV) like $V_\gamma 9$ (subgroup II) probably has no *Kpn*I site in its sequence. However, a *Kpn*I site could be detected 4.3 kb from 5′ of $V_\gamma 11$ (unpublished data and Figure 7). If our previous argument about the spacing of the V_γ genes and the presence of a *Kpn*I site in most V_γ genes is again true, this could mean that a new gene is present between $V_\gamma 10$ and $V_\gamma 11$. No rearranged fragment sizes corresponding to this suspected gene have been detected so far in leukaemia samples and, therefore, if the presence of this gene is confirmed, it is probably a pseudogene (designated $\psi V_\gamma B$ in the map Figure 7, by analogy with $\psi V_\gamma A$, the pseudogene found 6 kb in 5′ of $V_\gamma 9$) which,

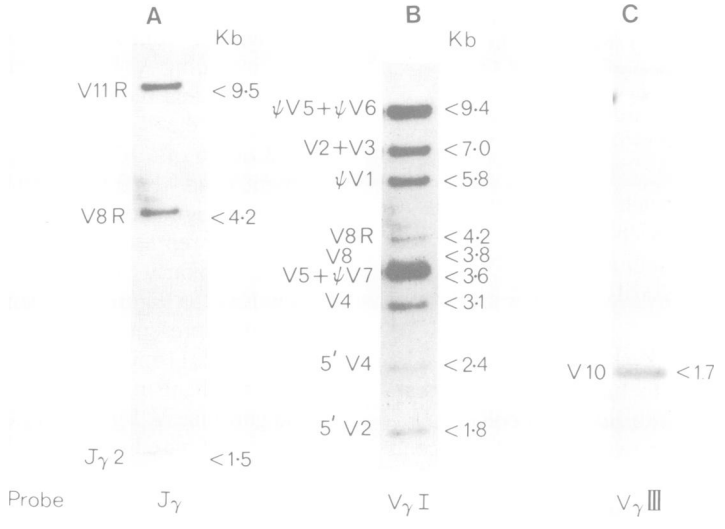

Fig. 5. Identification of V_γIV subgroup gene rearranged in JM DNA. Southern filter hybridization of JM genomic DNA digested wtih *Eco*RI and hybridized with J_γ probe M13H60 (**A**), V_γI probe (**B**), V_γIII probe (**C**) (see Figure 1 and text). Each line represents a different gel run and sizes plus designations for each band are indicated. These designations are based on sizes of cloned DNA fragments (Lefranc *et al.*, 1986b). Genes $V_\gamma 2$ and $V_\gamma 4$ contain *Eco*RI sites so that they appear as two hybridizing bands. The $V_\gamma 4$ germline does not exist as a clone and the assignment is to the only remaining fragment, 3.1 kb. This assignment is almost certain because T-cell lines with rearranged $V_\gamma 4$ and $V_\gamma 5$ possess the 3.1-kb band but those with both $V_\gamma 4$ alleles rearranged do not have this band. The intense 3.6-kb band is a doublet containing $\psi V_\gamma 7$ and a new gene; this band can be separated into two bands on long runs (unpublished data), and in this case, the upper band corresponds to the new gene, $V_\gamma 5$, located between $V_\gamma 4$ and $\psi V_\gamma 5$. This gene belongs to subgroup I.

along with the other pseudo V_γ genes are rarely found to be rearranged [with the possible exception of $\psi V_\gamma 7$ (Chen *et al.*, 1987; Le Paslier *et al.*, 1987)].

Discussion

This paper describes a new V_γ gene ($V_\gamma 10$) belonging to a new subgroup of which this gene appears to be the sole member. Two other subgroups having previously been described (Lefranc *et al.*, 1986b) the $V_\gamma 10$ gene belongs to a subgroup we have designated $V_\gamma III$. A partial sequence of the Jurkatt rearranged V_γ gene (Quertermous *et al.*, 1986) and rearrangement data above imply another, distinct subgroup, $V_\gamma IV$. This nomenclature has logic paralleled to the classification of the immunoglobulin subgroups (Kabat *et al.*, 1983) and to the probable order of the V_γ subgroups in the genome (i.e. $V_\gamma I - V_\gamma IV$) (see above). The sum total of known human V_γ genes is summarized in Table I. The probable order of these subgroups is $V_\gamma I - V_\gamma II - V_\gamma III - V_\gamma IV - C_\gamma$ with there being a minimum of nine genes in $V_\gamma I$ but probably only single genes in subgroups $V_\gamma II$, III and IV. This organization is illustrated in Figure 7. It is possible that other infrequently rearranged V_γ gene will be found but the limited number of V_γ gene rearrangements observed in unfractionated thymus cells (Lefranc *et al.*, 1986b) or in leukaemia cell lines (Lefranc and Rabbitts, 1985; Murre *et al.*, 1985) argues that those giving functional γ protein are limited to the few so far described, viz. eight V_γ genes.

Since the majority of rearrangements occur to either of two J_γ segments adjacent to either $C_\gamma 1$ or $C_\gamma 2$ (Lefranc *et al.*, 1986a) and although other J_γ segments do occur (in preparation for publication), it is now possible to decide in the vast majority of cases which V_γ gene is rearranged in a T-cell clone, cell line or tumour sample simply by determination of the size of the rearranged fragments. This may be clinically useful in deciding clonality of leukaemia samples and provides an easy way to determine V_γ gene usage. The various rearranged fragment sizes expected are given in Table II. Table IIA gives the sizes of

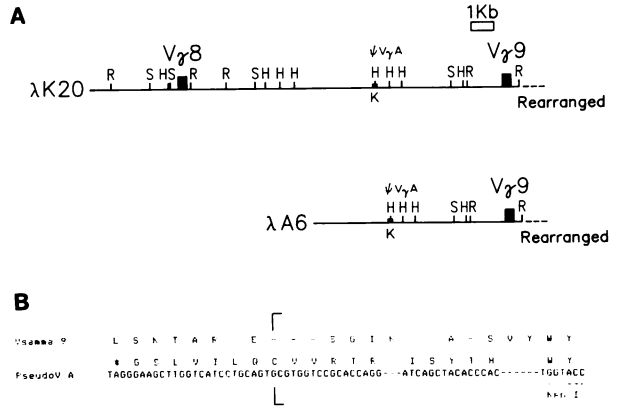

Fig. 6. Location of pseudogene $V_\gamma A$. (**A**) Restriction maps of λ K20 and λ A6 indicating positions of $V_\gamma 9$, $\psi V_\gamma A$ and the *Kpn*I site within $\psi V_\gamma A$ (this is the only *Kpn*I site shown). R, *Eco*RI; S, *Sac*I; H, *Hin*dIII; K, *Kpn*I. (**B**) Nucleotide sequence and deduced protein sequence immediately upstream of the *Kpn*I in $\psi V_\gamma A$; note the stop codon (indicated by * at the N terminal of this sequence). On the top line is the comparable protein sequence of $V_\gamma 9$, with dashes indicating identical residues. The intra-chain disulphide bond is indicated.

Table I. Human TRG V_γ subgroups

Gene	Subgroup	Reference
$V_\gamma 2$	$V_\gamma I$	Lefranc *et al.* (1986b)
$V_\gamma 3$		Lefranc *et al.* (1986b,c)
$V_\gamma 4$		Lefranc *et al.* (1986b)
$V_\gamma 5$		This paper
$V_\gamma 8$		Lefranc *et al.* (1986b)
$\psi V_\gamma 1$		Lefranc *et al.* (1986b)
$\psi V_\gamma 5$		Lefranc *et al.* (1986b)
$\psi V_\gamma 6$		Lefranc *et al.* (1986b)
$\psi V_\gamma 7$		Lefranc *et al.* (1986b)
$V_\gamma 9$	$V_\gamma II$	Lefranc *et al.* (1986b,c)
$V_\gamma 10$	$V_\gamma III$	This paper
$V_\gamma 11$	$V_\gamma IV$	This paper

No subgroup has been assigned to $\psi V_\gamma A$ since no functional gene nor rearrangement have been described so far for this gene.

Table IIA. Size of restriction fragments for unrearranged human J_γ segments

Segment	*Bam*HI	*Hin*dIII	*Eco*RI	*Sac*I
$J_\gamma 1$	20	2.1	1.5	6.4
$J_\gamma 2$	13.0	2.1[a]	3.2[a]	7.0
		5	1.5	

Sizes are derived from the analysis of cloned DNA segments previously described (Lefranc *et al.*, 1986b).
[a]An RFLP occurs with $J_\gamma 2$ with these enzymes (Lefranc and Rabbitts, 1985; Lefranc *et al.*, 1986a). As a consequence of this polymorphism and because of this existence of the J_γ segments (e.g., $J_\gamma P$) (Lefranc *et al.*, 1986b) *Bam*HI is the most useful enzyme for detection of rearrangements.

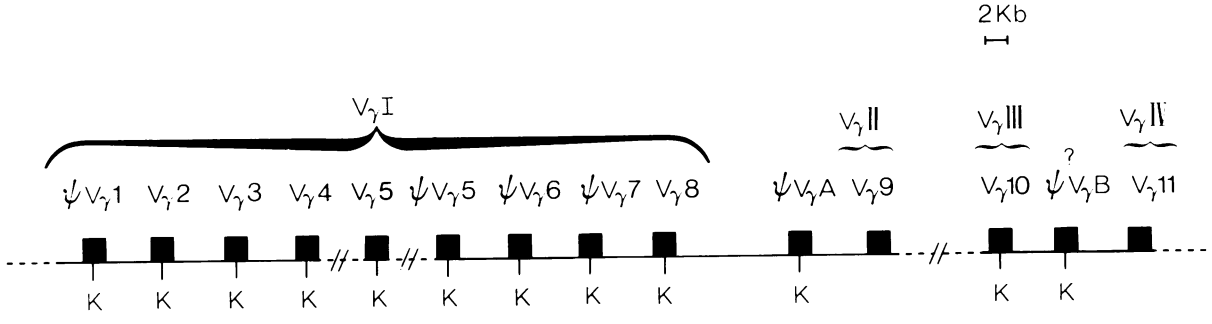

Fig. 7. Diagrammatic representation of the human TRG V_γ locus. The estimated order of V_γ genes and spatial organization of the human TRG γ locus. Each individual V_γ gene is assigned to its subgroup as indicated. K, *Kpn*I.

Table IIB. Size of rearranged fragments (kb) when V_γ genes are joined to $J_\gamma1$ or $J_\gamma2$

Subgroup	Gene	*Bam*HI	*Hin*dIII	*Eco*RI
I	$V_\gamma2$	11.7	4.3	0.9
	$V_\gamma3$	16	3.7	5.4 ($+V_\gamma2$)
	$V_\gamma4$	21	4.3	0.9
	$V_\gamma5$[a]	~25	3.6	2.2
	$\psi V_\gamma7$[a]	~35	2.9	3.1
	$V_\gamma8$	~40	2.9	4.2
II	$V_\gamma9$	15.5	4.0	2.4
III	$V_\gamma10$	19	5.1	0.65
IV	$V_\gamma11$	12	5.6	9.5

Predicted sizes of rearranged restriction fragments resulting from rearrangement of human TRG V_γ segments. Sizes are derived from the analysis of cloned DNA segments for subgroups I and II (Lefranc *et al.*, 1986b) and subgroup III (this paper) and deducted from rearrangement patterns observed in JM/Jurkatt cells for subgroup IV (our own unpublished data and Quertermous *et al.*, 1986).
[a]Sizes for $V_\gamma5$ (a new gene identified by deletion mapping of rearranged T-cell lines) and $\psi V_\gamma7$ have been deduced by the rearrangement sizes observed in some leukaemia cells (Chen *et al.*, 1987; Le Paslier *et al.*, 1987).

unrearranged J_γ segments and Table IIB the various sizes expected for rearrangement of each V_γ gene. The use of a suitable set of restriction enzymes (Table IIB) would therefore make an unequivocal assignment of V_γ rearrangement possible.

Materials and methods

λ *phage isolation and mapping*

Rearranged TRG γ genes were isolated from a *Sau*3A partial digest genomic library of RPMI 8402 (R.Baer, T.Boehm and T.H.Rabbitts, in preparation) using a $J_\gamma1$ probe [this probe, pH60, containing the 700-bp *Hin*dIII–*Eco*RI from M13H60 (Lefranc and Rabbitts, 1985) subcloned in pUC9]. Phage were mapped by standard procedures and relevant subclones prepared (see text) in pUC or M13 vector (Vieira and Messing, 1982).

Hybridization and sequencing procedure

Southern filter hybridization (Southern, 1975) was carried out with 10 μg genomic DNA using nick-translated probes (Rigby *et al.*, 1977). Conditions for hybridization, washing and monitoring are previously described (Lefranc *et al.*, 1986b).

DNA sequencing was carried out using M13 vector and dideoxy chain termination methods (Sanger *et al.*, 1977; Bankier and Barrell, 1983). Sequences were aligned by computer comparison (Staden, 1986).

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Note added in proof

A genomic clone isolated from the JM cell line contained rearranged $V_\gamma11$ and unrearranged $V_\gamma10$. This confirms the location of $V_\gamma11$ at 8.5 kb downstream of $V_\gamma10$ as well as the presence of pseudo $V_\gamma B$.