

# Rearrangement of Variable Region T Cell Receptor $\gamma$ Genes in Acute Lymphoblastic Leukemia

## V $\gamma$ Gene Usage Differs in Mature and Immature T Cells

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### Abstract

Using probes recognizing variable regions (V $\gamma$ ) and joining regions (J $\gamma$ ) of the T cell receptor (TCR)  $\gamma$  gene, we have analyzed the usage of V $\gamma$  genes in 24 patients with T cell acute lymphoblastic leukemia (ALL) and 36 patients with B-precursor ALL. In CD3<sup>-</sup> T-ALL derived from immature T cells, V $\gamma$  genes more proximal to J $\gamma$  were frequently rearranged; V $\gamma$ 8, V $\gamma$ 9, V $\gamma$ 10, and V $\gamma$ 11 were used in 19 of 24 rearrangements. In contrast, CD3<sup>+</sup> T-ALL derived from a more mature stage of T cell ontogeny, showed a high frequency of rearrangements involving V $\gamma$  genes distal to J $\gamma$ ; V $\gamma$ 2, V $\gamma$ 3, V $\gamma$ 4, and V $\gamma$ 5 were used in 17 of 25 rearrangements. In B-precursor ALL, no notable bias of V $\gamma$  gene usage was observed. This probably reflects the possibility that TCR genes may not rearrange according to a T cell hierarchy when under control of a B cell gene program. Furthermore, deletions of those V $\gamma$  genes located 3' to rearranged V $\gamma$  genes were observed in all patients analyzed. This supports the theory that loop deletion is a major mechanism for TCR- $\gamma$  gene rearrangement.

### Introduction

In addition to the T cell receptor (TCR)<sup>1</sup> $\alpha$  and  $\beta$  genes, a third gene,  $\gamma$ , has been shown to undergo somatic rearrangement in T cells (1, 2). Similar to other rearranging genes, the TCR- $\gamma$  gene is composed of variable (V), joining (J), and constant (C) regions (2, 3). The number of V $\gamma$  genes is much smaller than observed with other rearranging genes; to date 14 V $\gamma$  genes, including 6 pseudogenes have been identified upstream to J $\gamma$  and C $\gamma$  (4). These V $\gamma$  genes are divided into four families (V $\gamma$ I, V $\gamma$ II, V $\gamma$ III, and V $\gamma$ IV) (4) and are shared by two sets of J $\gamma$  and C $\gamma$  genes that show a strong homology to each other (2, 3, 5).

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Received for publication 29 August 1988 and in revised form 18 November 1988.

1. Abbreviations used in this paper: ALL, acute lymphoblastic leukemia; C, constant region; J, joining region; TCR, T cell receptor; V, variable region.

J. Clin. Invest.

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0021-9738/89/04/1277/07 \$2.00

Volume 83, April 1989, 1277-1283

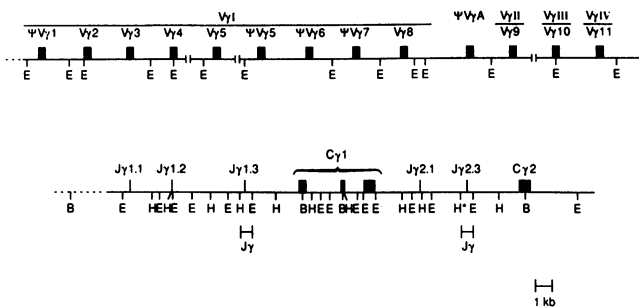
The protein product of the TCR- $\gamma$  gene has been shown to form a T cell receptor in association with the CD3 molecules on the cell surface and may be involved in some types of natural killer function (6-9). Although the specific roles of this TCR and the T cell subset expressing this receptor are still obscure, in general the TCR- $\gamma$  gene along with the other putative TCR genes probably encode a diverse set of receptor proteins involved in antigen recognition (8, 9).

In previous studies, we and others demonstrated a high incidence of TCR- $\gamma$  gene rearrangement not only in T cell acute lymphoblastic leukemia (T-ALL) but also in B-precursor ALL (10-12). In this study we have used probes recognizing V $\gamma$  and J $\gamma$ , and have analyzed the usage of V $\gamma$  genes in 24 T-ALL and 36 B-precursor ALL. V $\gamma$  genes more proximal to J $\gamma$  were frequently used in T-ALL derived from immature cells. In contrast, T-ALL derived from more mature T lineage cells demonstrated a high frequency of rearrangements involving V $\gamma$  genes more distal to J $\gamma$  and the most upstream (distal) functional V $\gamma$  gene showed the highest frequency of rearrangement. In B-precursor ALL, no particular clustering was observed. This observation may suggest that TCR- $\gamma$  gene rearrangement in B-precursor ALL is adventitious and does not follow the hierarchy observed in T lineage cells. Furthermore, deletions of V $\gamma$  genes located downstream to rearranged V $\gamma$  genes were observed in all patients analyzed, indicating loop deletion (13, 14) as the main mechanism for TCR- $\gamma$  gene rearrangement.

### Methods

**Cell samples.** Mononuclear cells were obtained from bone marrow by Ficoll-Hypaque gradient centrifugation at the time of diagnosis and before initiation of treatment. The samples evaluated contained > 90% malignant cells. Reactivity of malignant cells with a panel of MAbs was assessed by indirect immunofluorescence (15). The diagnosis of T-ALL was based on the expression of the T cell-associated antigens CD1, CD2, CD3, CD4, CD5, CD7, and CD8. T-ALL were divided into two subgroups according to CD3 expression (Table I). The diagnosis of B-precursor ALL was based on the expression of the B-cell-associated antigens CD19 and/or CD20 and the lack of surface Ig and T cell and myeloid-associated antigens. The B-precursor ALL studied here fell into two subgroups (16) (Table II): group III, Ia<sup>+</sup>, CD19<sup>+</sup>, and CD10<sup>+</sup>; and group IV, Ia<sup>+</sup>, CD19<sup>+</sup>, CD10<sup>+</sup>, and CD20<sup>+</sup>.

**Southern blot analysis.** High molecular weight DNA was extracted by standard procedures from the mononuclear cells and the DNA samples were digested with restriction endonucleases. The digested DNA was subjected to electrophoresis in agarose gels and transferred to nylon membranes. DNA samples bound to membranes were then hybridized to probes labeled with <sup>32</sup>P by the random primer method (17). Membranes were hybridized sequentially after removal of hybridized probes. Complete removal was confirmed by a test exposure before the next hybridization.



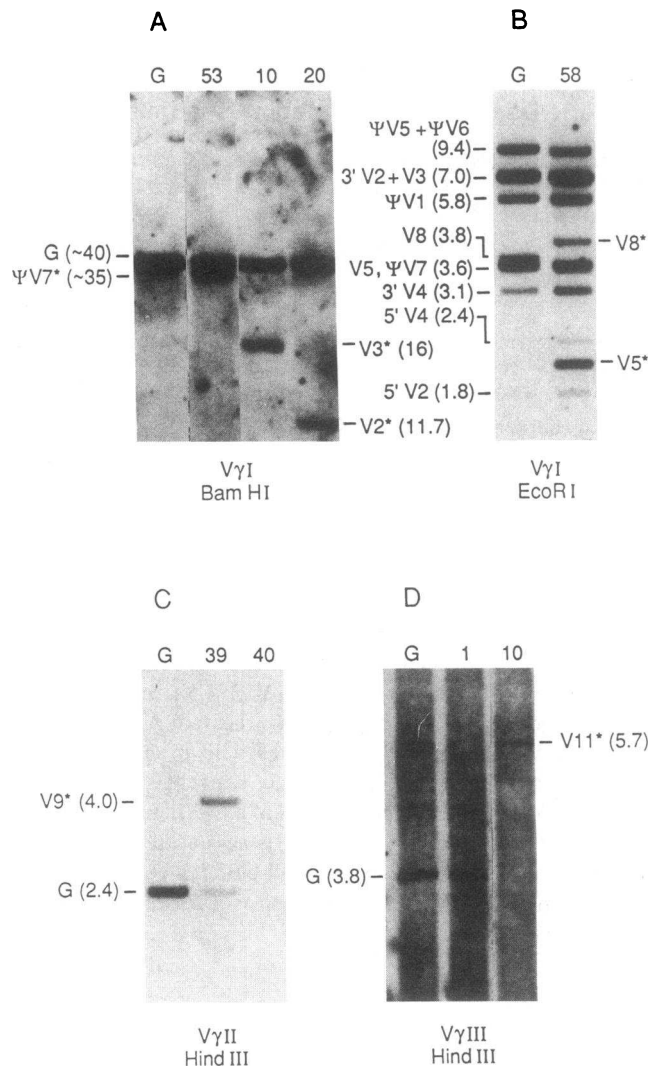
**Figure 1.** (Top) Diagram of V $\gamma$  region. E denotes Eco RI restriction sites. (Bottom) Restriction map of J $\gamma$  and C $\gamma$  region and the J $\gamma$  probe used in this study. B, E, and H denote Bam HI, Eco RI, and Hind III restriction sites, respectively. H\* indicates a polymorphic Hind III restriction site.

**Gene probes.** A restriction map of the TCR $\gamma$  region is presented in Fig. 1 and was derived from data presented in references 3, 4, and 18. The J $\gamma$  probe used in this study was a 0.7-kb Eco RI-Hind III genomic fragment provided by Dr. T. H. Rabbits (2). The J $\gamma$  probe hybridized to J $\gamma$ 1.3 and J $\gamma$ 2.3 but not to J $\gamma$ 1.1, J $\gamma$ 1.2, and J $\gamma$ 2.1 (Fig. 1) (18). The V $\gamma$  probes used in this study, provided by Dr. T. W. Mak, were as follows: V $\gamma$ I (360-bp Eco RI-Ava II fragment of cDNA HGPO3 [19]), V $\gamma$ II (290-bp Eco RI-Acc I fragment of cDNA HGPO2 (19); V $\gamma$ III (90-bp Eco RI-Apa I fragment of cDNA HGPO6 [19]). The V $\gamma$ I and V $\gamma$ II probes contained complete sequences of the V $\gamma$ I and V $\gamma$ II genes, respectively. The V $\gamma$ III probe contained only that portion 3' to the Eco RI site located in the V $\gamma$ III gene. The V $\gamma$ I probe hybridized to all V $\gamma$ I family members ( $\Psi$ V $\gamma$ 1, V $\gamma$ 2, V $\gamma$ 3, V $\gamma$ 4, V $\gamma$ 5,  $\Psi$ V $\gamma$ 5,  $\Psi$ V $\gamma$ 6,  $\Psi$ V $\gamma$ 7, and V $\gamma$ 8) and detected eight bands in Eco RI digests of germline DNA (4). Designations for each band are shown in Fig. 6. The V $\gamma$ I probe detected a single germline band after Bam HI digestion indicating that all V $\gamma$ I genes lie within a single Bam HI fragment. After hybridization with the V $\gamma$ II probe, Bam HI, Eco RI, and Hind III digests yielded single germline bands. Of these, Eco RI and Hind III bands contained only V $\gamma$ 9. The V $\gamma$ III probe detected single germline bands after Bam HI, Eco RI, and Hind III digestion. Hind III bands contained only V $\gamma$ 10, but Bam HI and Eco RI bands contained both V $\gamma$ 9 and V $\gamma$ 10, and V $\gamma$ 10 and V $\gamma$ 11, respectively. The order of genomic V $\gamma$  genes is shown in Fig. 1 (4, 20, 21). Rearrangements to V $\gamma$ 11 may represent rearrangements of  $\Psi$ V $\gamma$ B (4) since they localize to the same Eco RI fragment. Based on the sizes of the rearranged fragments, no rearrangements of  $\Psi$ V $\gamma$ B were found.

## Results

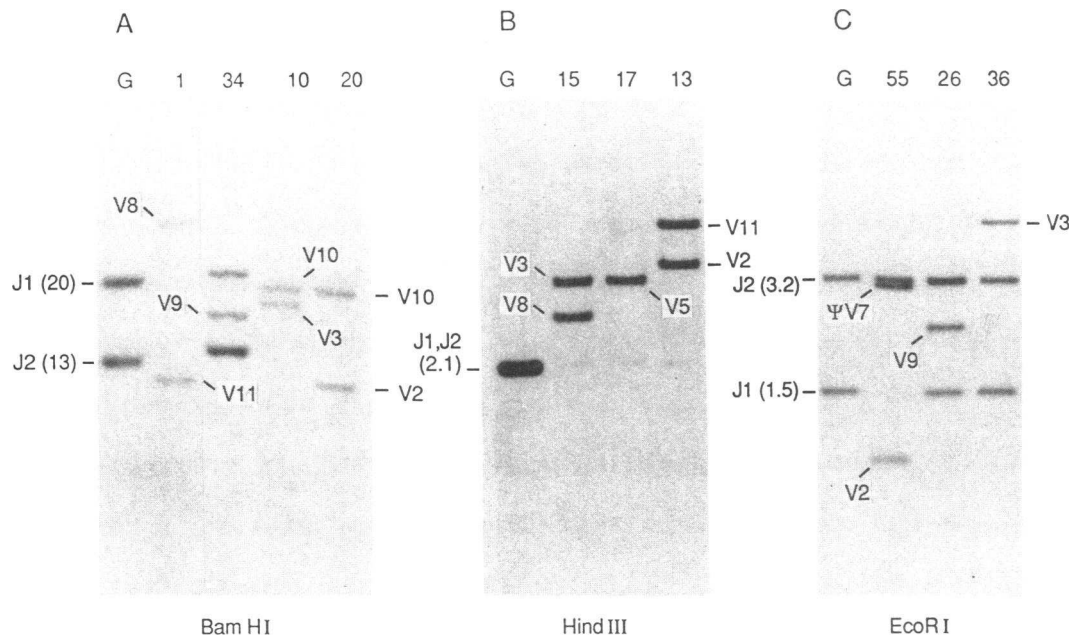
**Analysis of TCR- $\gamma$  gene rearrangement.** Fig. 2 demonstrates how the various V $\gamma$  rearrangements are recognized. In Fig. 2 A, members of the V $\gamma$ 1 family are recognized using Bam HI and the V $\gamma$ I probe. Rearrangements of the V2, V3, and  $\Psi$ V7 genes are recognized using this enzyme. Fig. 2 B shows that rearrangements of the V4, V5, and V8 genes are recognized using Eco RI and the V $\gamma$ I probe. Fig. 2 C demonstrates detection of rearrangement of V $\gamma$ 9, the only member described for the V $\gamma$ II family using Hind III and the V $\gamma$ II probe. Finally, Fig. 2 D uses Hind III and the V $\gamma$ III probe to recognize rearrangement of V $\gamma$ 10, the only member as yet described for the V $\gamma$ III family.

Fig. 3 demonstrates recognition of rearranged J $\gamma$  genes using the J $\gamma$  probe and the three restriction enzymes. As described by Forster et al. (4), those V $\gamma$  genes rearranged to J $\gamma$ 1.3 or J $\gamma$ 2.3 in a particular cell were assigned based on sizes of



**Figure 2.** Representative rearrangement patterns of the V $\gamma$  genes. The patient numbers are noted above each lane. Lane G represents the germline DNA control. The germline position and size of the individual bands recognized are indicated by bars at the side of each panel. (A and B) Bam HI and Eco RI digests, respectively, probed with the V $\gamma$ I probe. C is a Hind III digest probed with the V $\gamma$ II probe. D shows a Hind III digest probed with the V $\gamma$ III probe. Specific rearranged bands are indicated by \*.

rearranged J $\gamma$  bands after separate digestion with three enzymes (Bam HI, Eco RI, and Hind III) and using a J $\gamma$  probe. When V $\gamma$  genes were rearranged to J $\gamma$  genes other than J $\gamma$ 1.3 or J $\gamma$ 2.3, the usage of J $\gamma$  and V $\gamma$  genes was estimated by hybridization patterns obtained using specific V $\gamma$  probes. For each representative patient, rearranged J $\gamma$  bands are indicated by a line and the particular V $\gamma$  gene that has rearranged to that J $\gamma$  is given next to that line. Results presented in Figs. 2 and 3, in conjunction with numerous other experiments, confirm the published results (4) that V $\gamma$  and J $\gamma$  rearrangements are comparatively limited in number and can be assigned on the basis of size. The data also indicate that of six pseudo V $\gamma$  genes,  $\Psi$ V $\gamma$ 7 seems most capable of being rearranged in T cell or B-precursor acute lymphoblastic leukemia. Results described in this paper used these procedures to investigate V $\gamma$  and J $\gamma$  gene rearrangement in acute lymphoblastic leukemia. In pre-



**Figure 3.** Representative rearrangement patterns of  $J\gamma$  genes. Patient numbers are noted above each lane. Lane G shows the germline control, and the germline position and estimated size are indicated at the left of each panel. Specific rearranged bands for each patient are indicated by a small bar leading to the particular  $V\gamma$  gene which the size of the band suggests has rearranged in that patient as noted by Forster et al. (4). (A, B, and C) show DNA cut with Bam HI, Hind III, and Eco RI, respectively. All were probed with the  $J\gamma$  probe.

vious reports (10, 11) we demonstrated rearrangements of  $TCR\gamma$  in 24 of 24 T-ALL and 36 of 57 B-precursor ALL. In this study, we have analyzed  $V\gamma$  and  $J\gamma$  usage in these same patients. Results of these studies are summarized in Table I for T-ALL and Table II for B-precursor ALL. Data from the individual blots are shown only to make specific points.

All T-ALL and 16 B-precursor ALL had biallelic rearrangements and 20 B-precursor ALL had single allelic rearrangements of the  $TCR\gamma$  gene. In 22 T-ALL, germline bands corresponding to  $\gamma 1$  and  $\gamma 2$  loci were not detected after Bam HI, Eco RI, or Hind III digestion indicating rearrangements to  $J\gamma 2.3$  on both alleles (Table I). In contrast, the majority of B-precursor ALL (14 of 16 patients) with biallelic rearrangements retained  $\gamma 2$  in germline configuration. Both  $\gamma 1$  and  $\gamma 2$  germline bands were deleted in only 2 B-precursor ALL (Table II). Therefore, it seems that most rearrangements in B-precursor ALL occurred in the  $\gamma 1$  locus.

**Usage of  $V\gamma$  genes in  $CD3^-$  T-ALL.** The usage of individual  $V\gamma$  genes in T-ALL is summarized in Table I and Fig. 4. 12 patients were examined and these contained a total of 24 rearrangements. In  $CD3^-$  T-ALL, 19 of 24 rearrangements of the  $V\gamma$  genes were located proximal to  $J\gamma$ . The usage of  $V\gamma 8$  was most frequent; 9 of 24 rearrangements showed involvement of  $V\gamma 8$ .  $V\gamma 9$ , belonging to the  $V\gamma II$  family, was also frequently used;  $V\gamma 9$  was rearranged on five alleles. Rearrangements of  $V\gamma 10$  ( $V\gamma III$ ) and  $V\gamma 11$  ( $V\gamma IV$ ) were observed on two and three alleles, respectively. In contrast, involvement of  $V\gamma I$  genes located in an upstream region were less frequent;  $V\gamma 2$  and  $V\gamma 4$  rearranged on one allele each, and  $V\gamma 3$  rearranged on three alleles. Involvement of  $\Psi V\gamma 1$ ,  $\Psi V\gamma 5$ ,  $\Psi V\gamma 6$ ,  $\Psi V\gamma 7$ , and  $\Psi V\gamma A$  was not detected.

**Usage of  $V\gamma$  genes in  $CD3^+$  T-ALL.** The  $V\gamma$  gene usage in  $CD3^+$  T-ALL is summarized in Table I and Fig. 4. 12 patients were examined and these contained a total of 25 rearrangements. In contrast to the findings in  $CD3^-$  T-ALL,  $V\gamma I$  genes distal to  $J\gamma$  were rearranged in the majority of  $CD3^+$  T-ALL. Among a total of 25 rearrangements,  $V\gamma 2$  was most frequently used; eight alleles showed rearrangements of  $V\gamma 2$ .  $V\gamma 3$  and

$V\gamma 5$  were also frequently used; 3 and 5 alleles had rearrangements to  $V\gamma 3$  and  $V\gamma 5$ , respectively.  $V\gamma 4$  and  $\Psi V\gamma 7$  rearranged on a single allele, respectively, and  $V\gamma 8$  showed rear-

**Table I. Surface Antigen Expression and  $V\gamma$  Gene Usage in 24 Patients with T Cell Acute Lymphoblastic Leukemia**

Patient	Surface markers*					$V\gamma$ gene rearranged to $J\gamma$
	CD3	CD4	CD8	CD2	CD7	
1	-	-	-	-	+	$V\gamma 8, V\gamma 11$
2	-	-	-	+	+	$V\gamma 8, V\gamma 9$
3	-	-	-	+	ND	$V\gamma 8, V\gamma 9$
4	-	-	+	+	ND	$V\gamma 8, V\gamma 9$
5	-	-	+	+	ND	$V\gamma 3, V\gamma 8$
6	-	+	-	+	ND	$V\gamma 8, V\gamma 11$
7	-	+	+	+	ND	$V\gamma 3, V\gamma 8$
8	-	+	30	20	ND	$V\gamma 8, V\gamma 9$
9	-	+	+	+	ND	$V\gamma 2, V\gamma 4$
10	-	+	42	+	ND	$V\gamma 3, V\gamma 10$
11	-	+	+	+	ND	$V\gamma 10, V\gamma 11^\dagger$
12	-	ND	ND	+	+	$V\gamma 8, V\gamma 9$
13	+	+	-	-	+	$V\gamma 2, V\gamma 11$
14	+	-	20	+	ND	$V\gamma 4, \Psi V\gamma 7, V\gamma 9^\dagger$
15	+	40	40	+	ND	$V\gamma 3, V\gamma 8$
16	+	19	-	+	ND	$V\gamma 2, V\gamma 8$
17	+	10	+	+	ND	$V\gamma 5, V\gamma 5$
18	+	29	+	+	ND	$V\gamma 3, V\gamma 5$
19	+	+	+	+	ND	$V\gamma 2, V\gamma 8$
20	+	-	-	+	+	$V\gamma 2, V\gamma 10$
21	+	27	-	-	ND	$V\gamma 3, V\gamma 8$
22	+	42	45	+	ND	$V\gamma 2, V\gamma 5$
23	+	-	-	+	ND	$V\gamma 2, V\gamma 5$
24	+	+	-	+	ND	$V\gamma 2, V\gamma 2$

\* A minus sign denotes <10% positive cells and a plus sign >50% positive cells; numbers are specific percentages of positive cells.

† Rearrangements to  $J\gamma 1.1, J\gamma 1.2, \text{ or } J\gamma 2.1$ .

**Table II. Phenotype and V $\gamma$  Gene Usage in 36 Patients with B-Precursor Acute Lymphoblastic Leukemia**

Patient	Phenotype*	Germline <sup>†</sup>		V $\gamma$ gene rearranged to J $\gamma$	Patient	Phenotype	Germline <sup>†</sup>		V $\gamma$ gene rearranged to J $\gamma$
		$\gamma$ 1	$\gamma$ 2				$\gamma$ 1	$\gamma$ 2	
25	III	+	+	V $\gamma$ 4	43	IV	+	+	V $\gamma$ 3
26	III	+	+	V $\gamma$ 9	44	IV	+	+	V $\gamma$ 9
27	III	+	+	$\Psi$ V $\gamma$ 7	45	III	-	+	V $\gamma$ 5, $\Psi$ V $\gamma$ 7
28	III	+	+	V $\gamma$ 4	46	III	-	+	V $\gamma$ 4, V $\gamma$ 9
29	III	+	+	V $\gamma$ 2	47	III	-	+	V $\gamma$ 9, V $\gamma$ 9 <sup>§</sup>
30	III	+	+	V $\gamma$ 4	48	III	-	-	V $\gamma$ 2, V $\gamma$ 2
31	III	+	+	V $\gamma$ 5	49	III	-	+	V $\gamma$ 4, V $\gamma$ 9
32	III	+	+	V $\gamma$ 5	50	III	-	+	V $\gamma$ 2, V $\gamma$ 1 <sup>§  </sup>
33	III	+	+	V $\gamma$ 9	51	III	-	+	$\Psi$ V $\gamma$ 7, V $\gamma$ 9
34	III	+	+	V $\gamma$ 9	52	III	-	+	V $\gamma$ 2, V $\gamma$ 3
35	III	+	+	V $\gamma$ 9	53	III	-	+	$\Psi$ V $\gamma$ 7, V $\gamma$ 11
36	III	+	+	V $\gamma$ 3	54	III	-	+	V $\gamma$ 2, $\Psi$ V $\gamma$ 7
37	III	+	+	V $\gamma$ 9	55	III	-	+	V $\gamma$ 2, $\Psi$ V $\gamma$ 7
38	IV	+	+	V $\gamma$ 9	56	III	-	+	V $\gamma$ 11, V $\gamma$ 1 <sup>§  </sup>
39	IV	+	+	V $\gamma$ 8	57	III	-	+	V $\gamma$ 2, V $\gamma$ 5
40	IV	+	+	V $\gamma$ 4	58	III	-	-	V $\gamma$ 5, V $\gamma$ 8
41	IV	+	+	V $\gamma$ 9	59	III	-	+	V $\gamma$ 3, V $\gamma$ 4, V $\gamma$ 9
42	IV	+	+	V $\gamma$ 8	60	IV	-	+	V $\gamma$ 2, $\Psi$ V $\gamma$ 7

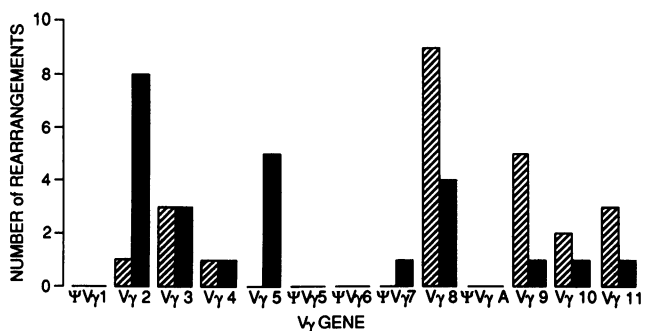
\* III: Ia<sup>+</sup>, CD19<sup>+</sup>, and CD10<sup>+</sup>; IV: Ia<sup>+</sup>, CD19<sup>+</sup>, CD10<sup>+</sup>, and CD20<sup>+</sup>. <sup>†</sup> Results after Bam HI digestion and hybridization with the J $\gamma$  probe. +, retention of germline bands; -, deletion of germline bands. <sup>§</sup> Rearrangements to J $\gamma$ 1.1, J $\gamma$ 1.2, or J $\gamma$ 2.1. <sup>||</sup> Rearrangements to one of the V $\gamma$ 1 family.

rangements on four alleles. V $\gamma$ 9 (V $\gamma$ II), V $\gamma$ 10 (V $\gamma$ III), and V $\gamma$ 11 (V $\gamma$ IV) were used on only one allele each.

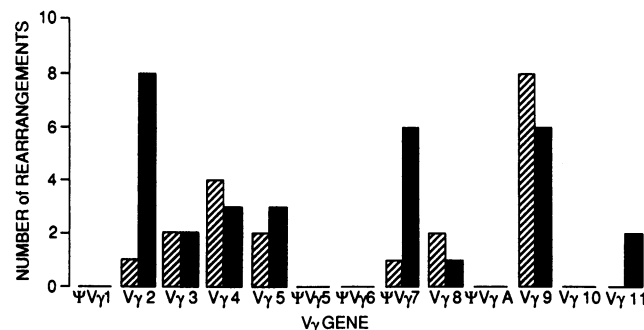
**Usage of V $\gamma$  genes in B-precursor ALL.** The usage of individual V $\gamma$  genes in B-precursor ALL is shown in Table II and Fig. 5. 57 patients with B-precursor ALL were examined of which 36 showed rearrangement of the TCR $\gamma$  gene. In these 36 patients, a total of 53 rearranged genes were observed. V $\gamma$ 2 and V $\gamma$ 9 were rearranged on a total of 9 and 14 alleles, respectively. Involvement of V $\gamma$ 3, V $\gamma$ 4, V $\gamma$ 5, and V $\gamma$ 8 were observed on four, seven, five, and three alleles, respectively. Rearrangements of  $\Psi$ V $\gamma$ 7 were also frequently observed; seven alleles showed involvement of  $\Psi$ V $\gamma$ 7. The usage of V $\gamma$ 11 (V $\gamma$ IV) was observed on two alleles. Rearrangements of V $\gamma$ 10 (V $\gamma$ III) and pseudogenes other than  $\Psi$ V $\gamma$ 7 were not observed.

Thus, no notable selectivity of V $\gamma$  gene usage was observed in B-precursor ALL. However, when these patients were subdivided into two subgroups; 20 patients with single allelic rearrangements and 16 patients with biallelic rearrangements, most V $\gamma$ 2 and  $\Psi$ V $\gamma$ 7 rearrangements were observed in patients with biallelic rearrangements.

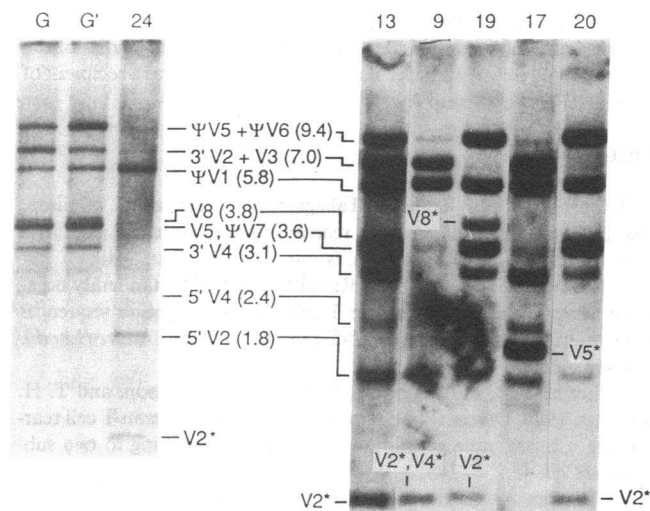
**Deletion pattern of V $\gamma$  genes.** Eco RI digests probed with V $\gamma$ I afford the opportunity to observe all of the V $\gamma$ I genes on the same blot. This was demonstrated above (Fig. 2 B). In addition to rearrangements, the complexity of the pattern facilitates detection of deletions that occur during rearrangement. To analyze the deletion pattern of V $\gamma$  genes, we examined the Eco RI digests that had been hybridized with the V $\gamma$ I and V $\gamma$ II probes in 12 patients with T-ALL. Bam HI and Hind



**Figure 4.** Usage of V $\gamma$  genes in 24 patients with T-ALL. Columns indicate the particular V $\gamma$  gene rearranged in 12 CD3<sup>-</sup> T-ALL (▨) and 12 CD3<sup>+</sup> T-ALL patients (■).



**Figure 5.** Usage of V $\gamma$  genes in 36 patients with B-precursor ALL. Figure indicates which V $\gamma$  gene rearranged in 20 patients with single allelic rearrangements (▨) and 16 patients with bi-allelic rearrangements (■).



**Figure 6.** Representative deletion patterns of V $\gamma$ I genes using Eco RI. The patient numbers are noted above each lane. Lane G and G' show the germline controls cut with Eco RI. The specific V $\gamma$  gene and the size (in kilobases) corresponding to each germline band are indicated by bars between blots. \*Rearranged V $\gamma$  genes.

III digests hybridized with the V $\gamma$ II and V $\gamma$ III probes were also examined. As illustrated in Fig. 6 and summarized in Table III, most patients showed deletion of V $\gamma$  genes intervening between the rearranged V $\gamma$  genes and the 3'-most terminus. These findings support the hypothesis that loop deletion (13, 14) is the primary mechanism for V $\gamma$  gene rearrangement. As described earlier (Tables I and II) Pt. 24 was found to be rearranged to V $\gamma$ 2 on both alleles, and Fig. 6 shows that Pt. 24 has deleted all the V region genes intervening between V $\gamma$ 2 and the constant region. Pt. 13 was found to be rearranged to V $\gamma$ 2 and V $\gamma$ 11, and therefore an apparent germline pattern is observed with this patient. This is solely due to the allele that rearranged to V $\gamma$ 11. Pt. 9 was shown to rearrange to V $\gamma$ 2 and V $\gamma$ 4, and Fig. 6 shows that all genes located 5' to V $\gamma$ 4 are in germline whereas those genes located between V $\gamma$ 4 and the

constant region have been deleted. A similar situation occurred with Pt. 17. Pt. 1 with rearrangements to V $\gamma$ 8 and V $\gamma$ 11 showed retention of all V $\gamma$ I, V $\gamma$ II, and V $\gamma$ III genes, and no germline V $\gamma$ IV (V $\gamma$ 11) genes identified by the V $\gamma$ III probe and Eco RI digestion.

Interesting hybridization patterns of the V $\gamma$ I probe were observed in three patients (Pts. 10, 19, and 20) with rearrangements involving V $\gamma$ 3 and V $\gamma$ 10, V $\gamma$ 2 and V $\gamma$ 8, and V $\gamma$ 2 and V $\gamma$ 10, respectively. In these patients, 7.0-kb bands corresponding to V $\gamma$ 2 and V $\gamma$ 3 and 2.4-kb bands corresponding to 5'V $\gamma$ 4 were lost with the retention of 9.4-kb bands corresponding to ΨV $\gamma$ 5 and ΨV $\gamma$ 6. Because the intensities of the 9.4-kb bands increased in spite of single allelic deletions of ΨV $\gamma$ 5 and ΨV $\gamma$ 6 caused by rearrangements to V $\gamma$ 2 or V $\gamma$ 3, it is possible that an Eco RI restriction site between V $\gamma$ 3 and V $\gamma$ 4 may be polymorphic and this polymorphic Eco RI fragment containing V $\gamma$ 2, V $\gamma$ 3, and 5'V $\gamma$ 4 may be overlaid on the normal 9.4-kb band. This possibility is supported by the findings of increased intensities of the 9.4-kb bands compared with the 7.0-kb bands in germline DNA (Fig. 6, lane G'), and by the lack of aberrant rearranged bands when samples cut with Hind III or Bam HI were probed.

## Discussion

Acute lymphoblastic leukemia results from the clonal expansion of cells arrested at different stages of lymphoid ontogeny (22, 23). These leukemic cells provide models for analyzing the molecular events that occur during lymphocyte development (10, 11, 15, 24–26). In this study, we have analyzed the usage of V $\gamma$  genes in patients with T-ALL and B-precursor ALL. Based on the expression of the CD3 antigen, the patients with T-ALL were divided into two subgroups: CD3<sup>-</sup> T-ALL cells presumably representing T lineage cells arrested early in ontogeny; CD3<sup>+</sup> T-ALL cells presumably derived from a more mature stage of T cell ontogeny. In CD3<sup>-</sup> T-ALL, rearrangements frequently involved V $\gamma$ 8 and V $\gamma$ 9, which are located proximal to J $\gamma$ , and rearrangements of V $\gamma$  genes located distal

**Table III.** Patterns of V $\gamma$  Gene Deletions in 12 Patients with T Cell Acute Lymphoblastic Leukemia

Patient	V $\gamma$ I								V $\gamma$ II <sup>‡</sup>	V $\gamma$ III <sup>§</sup>	Usage of V $\gamma$ genes
	ΨV $\gamma$ 1	5'V $\gamma$ 2	V $\gamma$ 2, V $\gamma$ 3	5'V $\gamma$ 4	V $\gamma$ 4	ΨV $\gamma$ 5, ΨV $\gamma$ 6	V $\gamma$ 5, ΨV $\gamma$ 7	V $\gamma$ 8	V $\gamma$ 9	V $\gamma$ 10	
1	+	+	+	+	+	+	+	+	+	+	V $\gamma$ 8, V $\gamma$ 11
8	+	+	+	+	+	+	+	+	R/D	-	V $\gamma$ 8, V $\gamma$ 9
9	+	+	+	+	-	-	-	-	-	-	V $\gamma$ 2, V $\gamma$ 4
10	+	+	-	-	+	+	+	+	+	R/-	V $\gamma$ 3, V $\gamma$ 10
13	+	+	+	+	+	+	+	+	+	+	V $\gamma$ 2, V $\gamma$ 11
15	+	+	+	+	+	+	+	-	-	-	V $\gamma$ 3, V $\gamma$ 8
17	+	+	+	+	+	-	-	-	-	-	V $\gamma$ 5, V $\gamma$ 5
19	+	+	-	-	+	+	+	+	-	-	V $\gamma$ 2, V $\gamma$ 8
20	+	+	-	-	+	+	+	+	+	R/-	V $\gamma$ 2, V $\gamma$ 10
22	+	+	+	+	+	-	-	-	-	-	V $\gamma$ 2, V $\gamma$ 5
23	+	+	+	+	+	-	-	-	-	-	V $\gamma$ 2, V $\gamma$ 5
24	+	+	-	-	-	-	-	-	-	-	V $\gamma$ 2, V $\gamma$ 2

+ , germline, - , deleted, and R, rearranged. \* Results after Eco RI digestion. ‡ Results after Eco RI and Hind III digestion. § Results after Hind III digestion.

to J $\gamma$  were less frequently observed. In contrast to the findings in CD3<sup>-</sup> T-ALL, leukemic cells from CD3<sup>+</sup> T-ALL frequently showed rearrangement of V $\gamma$ 2, which is the most upstream functional V $\gamma$  gene detected so far and in these cells, those V $\gamma$  genes located distal to J $\gamma$  (V $\gamma$ 2, V $\gamma$ 3, V $\gamma$ 4, and V $\gamma$ 5) were rearranged on the majority of alleles. In contrast to a recent publication (27), we were unable to detect a bias for or against usage of specific J $\gamma$  loci, even when cells displayed a decided bias for V $\gamma$  usage. This selectivity of V $\gamma$  gene usage is similar to that observed in Ig heavy chain gene rearrangements; V<sub>H</sub> genes proximal to J<sub>H</sub> are preferentially used in murine immature B lineage cells and during further differentiation, expression of V<sub>H</sub> genes proximal to J<sub>H</sub> is replaced by expression of V<sub>H</sub> genes located more 5' (distal) to J<sub>H</sub> (28, 29).

Others have observed that the proportion of rearrangements involving J $\gamma$  genes distal to C $\gamma$  was greater in immature T cells than mature cells (19). We have demonstrated similar findings for the TCR- $\alpha$  gene; immature T lineage cells preferentially used J $\alpha$  genes located proximal to V $\alpha$  genes and this was in sharp contrast to results using mature T lineage cells (11, 30). From work presented here, it appears that V $\gamma$  genes proximal to J $\gamma$  may initially rearrange to J $\gamma$  genes located distal to the C $\gamma$  region in the more immature T cells and during further differentiation the cells may successively rearrange using more 5' V $\gamma$  genes and 3' J $\gamma$  genes with concomitant deletion of the preexisting VJ $\gamma$  complex. It is also possible that 5' V $\gamma$  genes may undergo secondary rearrangements resulting in V $\gamma$  gene replacement without J $\gamma$  gene replacement in an analogous manner to that of the Ig heavy chain gene (31, 32). Because our conclusions are drawn from examination of leukemic cells, the possibility exists that the CD3<sup>-</sup> leukemic T cell does not represent the normal precursor for the CD3<sup>+</sup> T cell but are rather dead-end cells. Using the V $\gamma$  probes, deletions of V $\gamma$  genes located 3' to recombined V $\gamma$  genes were observed in all 12 patients analyzed. These findings suggest that loop deletion is the major mechanism for TCR- $\gamma$  gene rearrangement and for TCR- $\alpha$  and  $\beta$  genes (13, 14).

Leukemic cells from B-precursor ALL showed no notable preference of V $\gamma$  gene usage. When patients were subdivided based on single or biallelic rearrangements, most rearrangements to V $\gamma$ 2 were observed in cells with rearrangements on both alleles and are presumably more differentiated with respect to TCR- $\gamma$  gene rearrangement (11). Leukemic cells with rearrangements on single alleles rarely demonstrated rearrangement of V $\gamma$ 2 and rearrangements of V $\gamma$ 9 were the most frequent. However, unlike the case with T-ALL, definition of the stage of development by V region selection in B-precursor ALL does not coincide with definition of developmental stage using cell surface markers. It seems probable, therefore, that rearrangement of the TCR- $\gamma$  gene in B-precursor ALL does not follow the maturational hierarchy outlined above for TCR- $\gamma$  gene rearrangement in T-ALL.

## Acknowledgments

We are indebted to Dr. T. W. Mak and A. Goldstein for their help in carrying out the studies, to the members of the Division of Hematology/Oncology for providing patient specimens, and to Dr. G. Mills for helpful discussions. Dr. T. W. Mak and T. H. Rabbitts kindly provided the DNA probes.

This work was supported in part by the Medical Research Council (to E. W. Gelfand), by grant IM-510 from the American Cancer Soci-

ety (to S. H. Benedict) and by grant GM-40767091 from the National Institutes of Health (to S. H. Benedict). Dr. Gelfand is a Scholar of the Raymond and Beverly Sackler Foundation. Dr. Hara was a recipient of the Terry Fox Fellowship Award.

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