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Research Article

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Rearrangement of Variable Region T Cell Receptor γ Genes in Acute Lymphoblastic Leukemia

Vγ Gene Usage Differs in Mature and Immature T Cells

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Abstract

Using probes recognizing variable regions (Vγ) and joining regions (Jγ) of the T cell receptor (TCR) γ gene, we have analyzed the usage of Vγ genes in 24 patients with T cell acute lymphoblastic leukemia (ALL) and 36 patients with B-precursor ALL. In CD3⁻ T-ALL derived from immature T cells, Vγ genes more proximal to Jγ were frequently rearranged; Vγ8, Vγ9, Vγ10, and Vγ11 were used in 19 of 24 rearrangements. In contrast, CD3⁺ T-ALL derived from a more mature stage of T cell ontogeny, showed a high frequency of rearrangements involving Vγ genes distal to Jγ; Vγ2, Vγ3, Vγ4, and Vγ5 were used in 17 of 25 rearrangements. In B-precursor ALL, no notable bias of Vγ 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 Vγ genes located 3′ to rearranged Vγ genes were observed in all patients analyzed. This supports the theory that loop deletion is a major mechanism for TCR-γ gene rearrangement.

Introduction

In addition to the T cell receptor (TCR)[1] α and β genes, a third gene, γ, has been shown to undergo somatic rearrangement in T cells (1, 2). Similar to other rearranging genes, the TCR-γ gene is composed of variable (V), joining (J), and constant (C) regions (2, 3). The number of Vγ genes is much smaller than observed with other rearranging genes; to date 14 Vγ genes, including 6 pseudogenes have been identified upstream to Jγ and Cγ (4). These Vγ genes are divided into four families (VγI, VγII, VγIII, and VγIV) (4) and are shared by two sets of Jγ and Cγ genes that show a strong homology to each other (2, 3, 5).

The protein product of the TCR-γ 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-γ 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-γ 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 Vγ and Jγ, and have analyzed the usage of Vγ genes in 24 T-ALL and 36 B-precursor ALL. Vγ genes more proximal to Jγ 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 Vγ genes more distal to Jγ and the most upstream (distal) functional Vγ gene showed the highest frequency of rearrangement. In B-precursor ALL, no particular clustering was observed. This observation may suggest that TCR-γ gene rearrangement in B-precursor ALL is adventitious and does not follow the hierarchy observed in T lineage cells. Furthermore, deletions of Vγ genes located downstream to rearranged Vγ genes were observed in all patients analyzed, indicating loop deletion (13, 14) as the main mechanism for TCR-γ 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 MAb 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⁺, CD19⁺, and CD10⁺; and group IV, Ia⁺, CD19⁺, CD10⁺, and CD20⁺.

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 ^{32}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.

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

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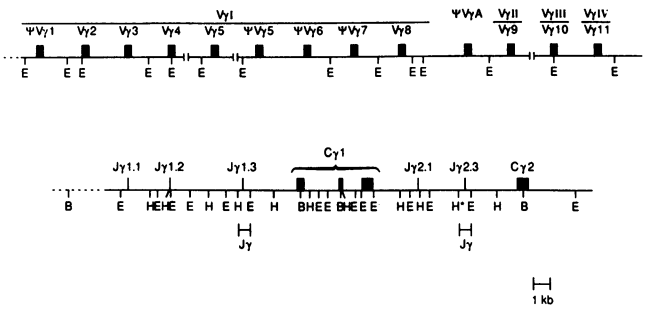

Figure 1. (*Top*) Diagram of Vγ region. E denotes Eco RI restriction sites. (*Bottom*) Restriction map of Jγ and Cγ region and the Jγ probe used in this study. B, E, and H denote Bam HI, Eco RI, and Hind III restriction sites, respectively. H* indicates a polymorphic Hind III restriction site.

Gene probes. A restriction map of the TCRγ region is presented in Fig. 1 and was derived from data presented in references 3, 4, and 18. The Jγ probe used in this study was an 0.7-kb Eco RI-Hind III genomic fragment provided by Dr. T. H. Rabbitts (2). The Jγ probe hybridized to Jγ1.3 and Jγ2.3 but not to Jγ1.1, Jγ1.2, and Jγ2.1 (Fig. 1) (18). The Vγ probes used in this study, provided by Dr. T. W. Mak, were as follows: VγI (360-bp Eco RI-Ava II fragment of cDNA HGPO3 [19]), VγII (290-bp Eco RI-Acc I fragment of cDNA HGPO2 (19); VγIII (90-bp Eco RI-Apa I fragment of cDNA HGPO6 [19]). The VγI and VγII probes contained complete sequences of the VγI and VγII genes, respectively. The VγIII probe contained only that portion 3′ to the Eco RI site located in the VγIII gene. The VγI probe hybridized to all VγI family members (ΨVγ1, Vγ2, Vγ3, Vγ4, Vγ5, ΨVγ5, ΨVγ6, ΨVγ7, and Vγ8) and detected eight bands in Eco RI digests of germline DNA (4). Designations for each band are shown in Fig. 6. The VγI probe detected a single germline band after Bam HI digestion indicating that all VγI genes lie within a single Bam HI fragment. After hybridization with the VγII probe, Bam HI, Eco RI, and Hind III digests yielded single germline bands. Of these, Eco RI and Hind III bands contained only Vγ9. The VγIII probe detected single germline bands after Bam HI, Eco RI, and Hind III digestion. Hind III bands contained only Vγ10, but Bam HI and Eco RI bands contained both Vγ9 and Vγ10, and Vγ10 and Vγ11, respectively. The order of genomic Vγ genes is shown in Fig. 1 (4, 20, 21). Rearrangements to Vγ11 may represent rearrangements of ΨVγB (4) since they localize to the same Eco RI fragment. Based on the sizes of the rearranged fragments, no rearrangements of ψVγB were found.

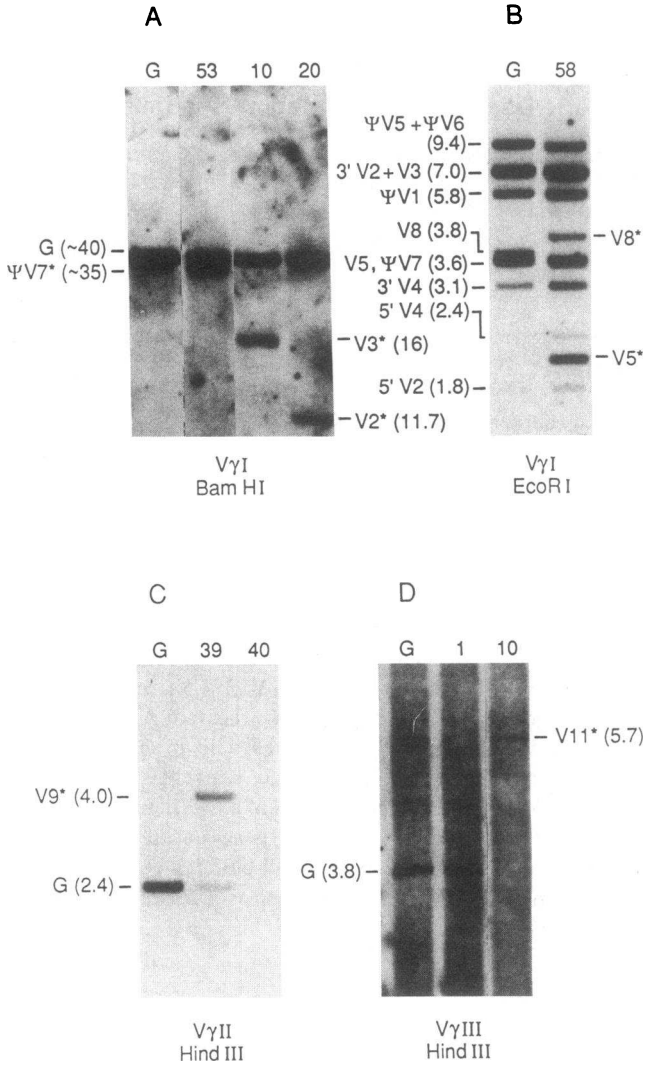

Figure 2. Representative rearrangement patterns of the Vγ genes. The patient numbers are noted above each lane. Lane *G* represents the germline DNA control. The germline position and size of the individual bands recognized are indicated by bars at the side of each panel. (*A* and *B*) Bam HI and Eco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

Results

Analysis of TCR-γ gene rearrangement. Fig. 2 demonstrates how the various Vγ rearrangements are recognized. In Fig. 2 *A*, members of the Vγ1 family are recognized using Bam HI and the VγI probe. Rearrangements of the V2, V3, and ΨV7 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 VγI probe. Fig. 2 *C* demonstrates detection of rearrangement of Vγ9, the only member described for the VγII family using Hind III and the VγII probe. Finally, Fig. 2 *D* uses Hind III and the VγIII probe to recognize rearrangement of Vγ10, the only member as yet described for the VγIII family.

Fig. 3 demonstrates recognition of rearranged Jγ genes using the Jγ probe and the three restriction enzymes. As described by Forster et al. (4), those Vγ genes rearranged to Jγ1.3 or Jγ2.3 in a particular cell were assigned based on sizes of rearranged Jγ bands after separate digestion with three enzymes (Bam HI, Eco RI, and Hind III) and using a Jγ probe. When Vγ genes were rearranged to Jγ genes other than Jγ1.3 or Jγ2.3, the usage of Jγ and Vγ genes was estimated by hybridization patterns obtained using specific Vγ probes. For each representative patient, rearranged Jγ bands are indicated by a line and the particular Vγ gene that has rearranged to that Jγ 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 Vγ and Jγ rearrangements are comparatively limited in number and can be assigned on the basis of size. The data also indicate that of six pseudo Vγ genes, ΨVγ7 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 Vγ and Jγ gene rearrangement in acute lymphoblastic leukemia. In pre-

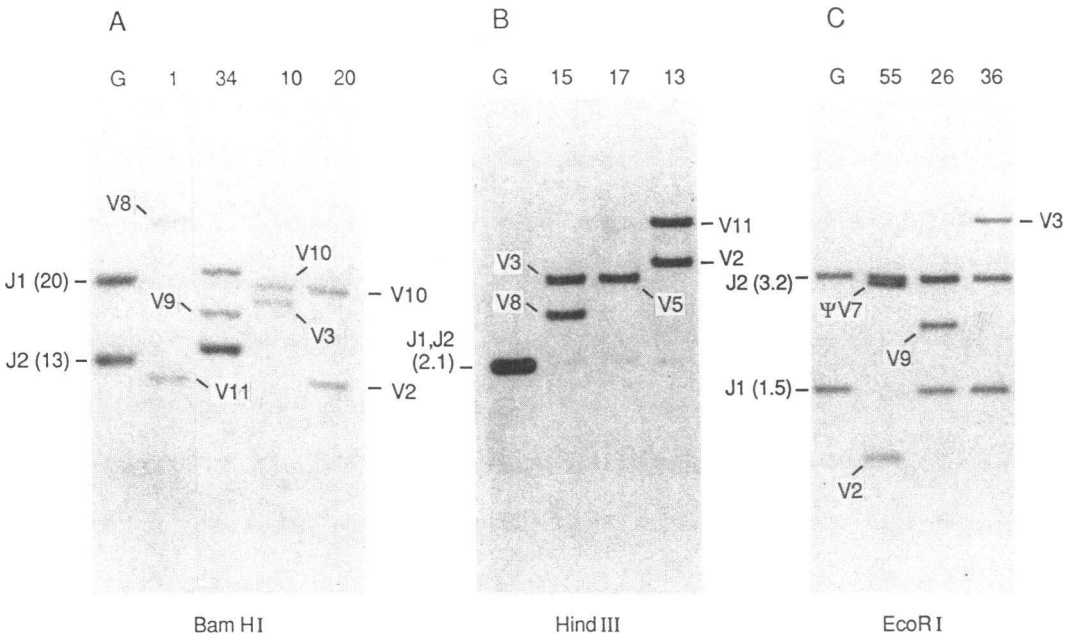

Figure 3. Representative rearrangement patterns of Jγ genes. Patient numbers are noted above each lane. Lane *G* shows the germline control, and the germline position and estimated size are indicated at the left of each panel. Specific rearranged bands for each patient are indicated by a small bar leading to the particular Vγ 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 Jγ probe.

vious reports (10, 11) we demonstrated rearrangements of TCRγ in 24 of 24 T-ALL and 36 of 57 B-precursor ALL. In this study, we have analyzed Vγ and Jγ 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-γ gene. In 22 T-ALL, germline bands corresponding to γ1 and γ2 loci were not detected after Bam HI, Eco RI, or Hind III digestion indicating rearrangements to Jγ2.3 on both alleles (Table I). In contrast, the majority of B-precursor ALL (14 of 16 patients) with biallelic rearrangements retained γ2 in germline configuration. Both γ1 and γ2 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 γ1 locus.

Usage of Vγ genes in CD3⁻ T-ALL. The usage of individual Vγ 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 Vγ genes were located proximal to Jγ. The usage of Vγ8 was most frequent; 9 of 24 rearrangements showed involvement of Vγ8. Vγ9, belonging to the VγII family, was also frequently used; Vγ9 was rearranged on five alleles. Rearrangements of Vγ10 (VγIII) and Vγ11 (VγIV) were observed on two and three alleles, respectively. In contrast, involvement of VγI genes located in an upstream region were less frequent; Vγ2 and Vγ4 rearranged on one allele each, and Vγ3 rearranged on three alleles. Involvement of ΨVγ1, ΨVγ5, ΨVγ6, ΨVγ7, and ΨVγA was not detected.

Usage of Vγ genes in CD3⁺ T-ALL. The Vγ 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, VγI genes distal to Jγ were rearranged in the majority of CD3⁺ T-ALL. Among a total of 25 rearrangements, Vγ2 was most frequently used; eight alleles showed rearrangements of Vγ2. Vγ3 and Vγ5 were also frequently used; 3 and 5 alleles had rearrangements to Vγ3 and Vγ5, respectively. Vγ4 and ΨVγ7 rearranged on a single allele, respectively, and Vγ8 showed rear-

Table I. Surface Antigen Expression and Vγ Gene Usage in 24 Patients with T Cell Acute Lymphoblastic Leukemia

	Surface markers*					
Patient	CD3	CD4	CD8	CD2	CD7	Vγ gene rearranged to Jγ
1	–	–	–	–	+	Vγ8, Vγ11
2	–	–	–	+	+	Vγ8, Vγ9
3	–	–	–	+	ND	Vγ8, Vγ9
4	–	–	+	+	ND	Vγ8, Vγ9
5	–	–	+	+	ND	Vγ3, Vγ8
6	–	+	–	+	ND	Vγ8, Vγ11
7	–	+	+	+	ND	Vγ3, Vγ8
8	–	+	30	20	ND	Vγ8, Vγ9
9	–	+	+	+	ND	Vγ2, Vγ4
10	–	+	42	+	ND	Vγ3, Vγ10
11	–	+	+	+	ND	Vγ10, Vγ11‡
12	–	ND	ND	+	+	Vγ8, Vγ9
13	+	+	–	–	+	Vγ2, Vγ11
14	+	–	20	+	ND	Vγ4, ΨVγ7, Vγ9‡
15	+	40	40	+	ND	Vγ3, Vγ8
16	+	19	–	+	ND	Vγ2, Vγ8
17	+	10	+	+	ND	Vγ5, Vγ5
18	+	29	+	+	ND	Vγ3, Vγ5
19	+	+	+	+	ND	Vγ2, Vγ8
20	+	–	–	+	+	Vγ2, Vγ10
21	+	27	–	–	ND	Vγ3, Vγ8
22	+	42	45	+	ND	Vγ2, Vγ5
23	+	–	–	+	ND	Vγ2, Vγ5
24	+	+	–	+	ND	Vγ2, Vγ2

* A minus sign denotes <10% positive cells and a plus sign >50% positive cells; numbers are specific percentages of positive cells.
‡ Rearrangements to Jγ1.1, Jγ1.2, or Jγ2.1.

Table II. Phenotype and Vγ Gene Usage in 36 Patients with B-Precursor Acute Lymphoblastic Leukemia

Patient	Phenotype*	Germline‡ γ1	Germline‡ γ2	Vγ gene rearranged to Jγ	Patient	Phenotype	Germline‡ γ1	Germline‡ γ2	Vγ gene rearranged to Jγ
25	III	+	+	Vγ4	43	IV	+	+	Vγ3
26	III	+	+	Vγ9	44	IV	+	+	Vγ9
27	III	+	+	ΨVγ7	45	III	−	+	Vγ5, ΨVγ7
28	III	+	+	Vγ4	46	III	−	+	Vγ4, Vγ9
29	III	+	+	Vγ2	47	III	−	+	Vγ9, Vγ9§
30	III	+	+	Vγ4	48	III	−	−	Vγ2, Vγ2
31	III	+	+	Vγ5	49	III	−	+	Vγ4, Vγ9
32	III	+	+	Vγ5	50	III	−	+	Vγ2, VγI§‖
33	III	+	+	Vγ9	51	III	−	+	ΨVγ7, Vγ9
34	III	+	+	Vγ9	52	III	−	+	Vγ2, Vγ3
35	III	+	+	Vγ9	53	III	−	+	ΨVγ7, Vγ11
36	III	+	+	Vγ3	54	III	−	+	Vγ2, ΨVγ7
37	III	+	+	Vγ9	55	III	−	+	Vγ2, ΨVγ7
38	IV	+	+	Vγ9	56	III	−	+	Vγ11, VγI§‖
39	IV	+	+	Vγ8	57	III	−	+	Vγ2, Vγ5
40	IV	+	+	Vγ4	58	III	−	−	Vγ5, Vγ8
41	IV	+	+	Vγ9	59	III	−	+	Vγ3, Vγ4, Vγ9
42	IV	+	+	Vγ8	60	IV	−	+	Vγ2, ΨVγ7

* III: Ia$^+$, CD19$^+$, and CD10$^+$, IV: Ia$^+$, CD19$^+$, CD10$^+$, and CD20$^+$. ‡ Results after Bam HI digestion and hybridization with the Jγ probe. +, retention of germline bands; −, deletion of germline bands. § Rearrangements to Jγ1.1, Jγ1.2, or Jγ2.1. ‖ Rearrangements to one of the VγI family.

rangements on four alleles. Vγ9 (VγII), Vγ10 (VγIII), and Vγ11 (VγIV) were used on only one allele each.

Usage of Vγ genes in B-precursor ALL. The usage of individual Vγ 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 TCRγ gene. In these 36 patients, a total of 53 rearranged genes were observed. Vγ2 and Vγ9 were rearranged on a total of 9 and 14 alleles, respectively. Involvement of Vγ3, Vγ4, Vγ5, and Vγ8 were observed on four, seven, five, and three alleles, respectively. Rearrangements of ΨVγ7 were also frequently observed; seven alleles showed involvement of ΨVγ7. The usage of Vγ11 (VγIV) was observed on two alleles. Rearrangements of Vγ10 (VγIII) and pseudogenes other than ΨVγ7 were not observed. Thus, no notable selectivity of Vγ 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 Vγ2 and ΨVγ7 rearrangements were observed in patients with biallelic rearrangements.

Deletion pattern of Vγ genes. Eco RI digests probed with VγI afford the opportunity to observe all of the VγI 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 Vγ genes, we examined the Eco RI digests that had been hybridized with the VγI and VγII probes in 12 patients with T-ALL. Bam HI and Hind

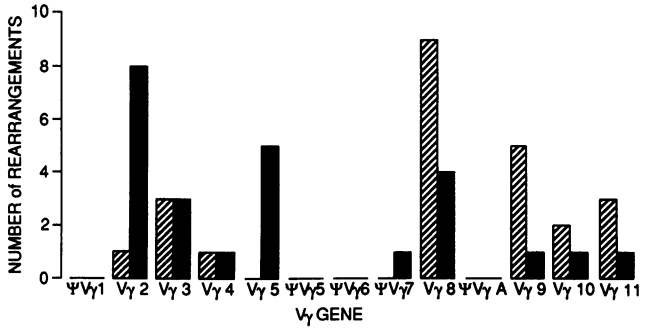

Figure 4. Usage of Vγ genes in 24 patients with T-ALL. Columns indicate the particular Vγ gene rearranged in 12 CD3$^-$ T-ALL (▨) and 12 CD3$^+$ T-ALL patients (■).

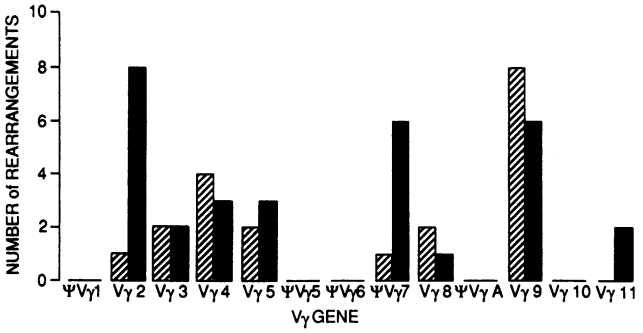

Figure 5. Usage of Vγ genes in 36 patients with B-precursor ALL. Figure indicates which Vγ gene rearranged in 20 patients with single allelic rearrangements (▨) and 16 patients with bi-allelic rearrangements (■).

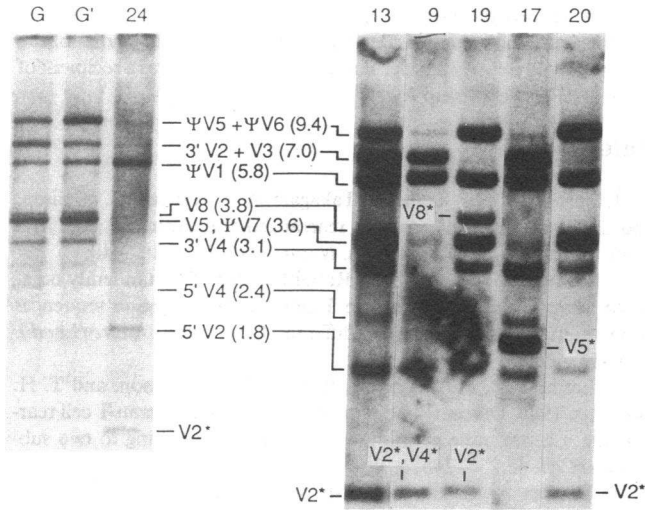

Figure 6. Representative deletion patterns of Vγ1 genes using Eco RI. The patient numbers are noted above each lane. Lane *G* and *G'* show the germline controls cut with Eco RI. The specific Vγ gene and the size (in kilobases) corresponding to each germline band are indicated by bars between blots. *Rearranged Vγ genes.

III digests hybridized with the VγII and VγIII probes were also examined. As illustrated in Fig. 6 and summarized in Table III, most patients showed deletion of Vγ genes intervening between the rearranged Vγ genes and the 3'-most terminus. These findings support the hypothesis that loop deletion (13, 14) is the primary mechanism for Vγ gene rearrangement. As described earlier (Tables I and II) Pt. 24 was found to be rearranged to Vγ2 on both alleles, and Fig. 6 shows that Pt. 24 has deleted all the V region genes intervening between Vγ2 and the constant region. Pt. 13 was found to be rearranged to Vγ2 and Vγ11, and therefore an apparent germline pattern is observed with this patient. This is solely due to the allele that rearranged to Vγ11. Pt. 9 was shown to rearrange to Vγ2 and Vγ4, and Fig. 6 shows that all genes located 5' to Vγ4 are in germline whereas those genes located between Vγ4 and the constant region have been deleted. A similar situation occurred with Pt. 17. Pt. 1 with rearrangements to Vγ8 and Vγ11 showed retention of all VγI, VγII, and VγIII genes, and no germline VγIV (Vγ11) genes identified by the VγIII probe and Eco RI digestion.

Interesting hybridization patterns of the VγI probe were observed in three patients (Pts. 10, 19, and 20) with rearrangements involving Vγ3 and Vγ10, Vγ2 and Vγ8, and Vγ2 and Vγ10, respectively. In these patients, 7.0-kb bands corresponding to Vγ2 and Vγ3 and 2.4-kb bands corresponding to 5'Vγ4 were lost with the retention of 9.4-kb bands corresponding to ΨVγ5 and ΨVγ6. Because the intensities of the 9.4-kb bands increased in spite of single allelic deletions of ΨVγ5 and ΨVγ6 caused by rearrangements to Vγ2 or Vγ3, it is possible that an Eco RI restriction site between Vγ3 and Vγ4 may be polymorphic and this polymorphic Eco RI fragment containing Vγ2, Vγ3, and 5'Vγ4 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 Vγ 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: $CD3^-$ T-ALL cells presumably representing T lineage cells arrested early in ontogeny; $CD3^+$ T-ALL cells presumably derived from a more mature stage of T cell ontogeny. In $CD3^-$ T-ALL, rearrangements frequently involved Vγ8 and Vγ9, which are located proximal to Jγ, and rearrangements of Vγ genes located distal

Table III. Patterns of Vγ Gene Deletions in 12 Patients with T Cell Acute Lymphoblastic Leukemia

	VγI								VγII‡	VγIII§	
Patient	ΨVγ1	5'Vγ2	Vγ2, Vγ3	5'Vγ4	Vγ4	ΨVγ5, ΨVγ6	Vγ5, ΨVγ7	Vγ8	Vγ9	Vγ10	Usage of Vγ genes
1	+	+	+	+	+	+	+	+	+	+	Vγ8, Vγ11
8	+	+	+	+	+	+	+	+	R/D	−	Vγ8, Vγ9
9	+	+	+	+	−	−	−	−	−	−	Vγ2, Vγ4
10	+	+	−	−	+	+	+	+	+	R/−	Vγ3, Vγ10
13	+	+	+	+	+	+	+	+	+	+	Vγ2, Vγ11
15	+	+	+	+	+	+	+	−	−	−	Vγ3, Vγ8
17	+	+	+	+	+	−	−	−	−	−	Vγ5, Vγ5
19	+	+	−	−	+	+	+	−	−	−	Vγ2, Vγ8
20	+	+	−	−	+	+	+	+	+	R/−	Vγ2, Vγ10
22	+	+	+	+	+	−	−	−	−	−	Vγ2, Vγ5
23	+	+	+	+	+	−	−	−	−	−	Vγ2, Vγ5
24	+	+	−	−	−	−	−	−	−	−	Vγ2, Vγ2

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

to Jγ were less frequently observed. In contrast to the findings in $CD3^-$ T-ALL, leukemic cells from $CD3^+$ T-ALL frequently showed rearrangement of Vγ2, which is the most upstream functional Vγ gene detected so far and in these cells, those Vγ genes located distal to Jγ (Vγ2, Vγ3, Vγ4, and Vγ5) 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 Jγ loci, even when cells displayed a decided bias for Vγ usage. This selectivity of Vγ gene usage is similar to that observed in Ig heavy chain gene rearrangements; V_H genes proximal to J_H are preferentially used in murine immature B lineage cells and during further differentiation, expression of V_H genes proximal to J_H is replaced by expression of V_H genes located more 5′ (distal) to J_H (28, 29).

Others have observed that the proportion of rearrangements involving Jγ genes distal to Cγ was greater in immature T cells than mature cells (19). We have demonstrated similar findings for the TCR-α gene; immature T lineage cells preferentially used Jα genes located proximal to Vα genes and this was in sharp contrast to results using mature T lineage cells (11, 30). From work presented here, it appears that Vγ genes proximal to Jγ may initially rearrange to Jγ genes located distal to the Cγ region in the more immature T cells and during further differentiation the cells may successively rearrange using more 5′ Vγ genes and 3′ Jγ genes with concomitant deletion of the preexisting VJγ complex. It is also possible that 5′ Vγ genes may undergo secondary rearrangements resulting in Vγ gene replacement without Jγ 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 $CD3^-$ leukemic T cell does not represent the normal precursor for the $CD3^+$ T cell but are rather dead-end cells. Using the Vγ probes, deletions of Vγ genes located 3′ to recombined Vγ genes were observed in all 12 patients analyzed. These findings suggest that loop deletion is the major mechanism for TCR-γ gene rearrangement and for TCR-α and β genes (13, 14).

Leukemic cells from B-precursor ALL showed no notable preference of Vγ gene usage. When patients were subdivided based on single or biallelic rearrangements, most rearrangements to Vγ2 were observed in cells with rearrangements on both alleles and are presumably more differentiated with respect to TCR-γ gene rearrangement (11). Leukemic cells with rearrangements on single alleles rarely demonstrated rearrangement of Vγ2 and rearrangements of Vγ9 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-γ gene in B-precursor ALL does not follow the maturational hierarchy outlined above for TCR-γ gene rearrangement in T-ALL.

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