T Cell Receptor δ Gene Rearrangements in Acute Lymphoblastic Leukemia

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Abstract

Using a newly isolated cDNA clone encoding the TCR-δ gene and genomic probes, we have analyzed T cell receptor (TCR) δ gene rearrangement in 19 patients with T cell acute lymphoblastic leukemia (T-ALL) and 29 patients with B-precursor ALL. Five out of seven CD3⁻ T-ALL and 4 of 12 CD3⁺ T-ALL showed bi-allelic rearrangements of the TCR-δ gene. In three CD3⁺ patients, a single allelic TCR-δ gene rearrangement was observed with rearrangement of the TCR-α gene on the other allele. In five CD3⁺ patients with bi-allelic rearrangements of the TCR-α gene, the TCR-δ gene locus was deleted. Transcription of the TCR-δ gene was also analyzed in six T-ALL. Five patients expressed TCR-δ transcripts. Only one T-ALL, presumably derived from the most immature T lineage cells, did not have TCR-δ transcripts, but expressed TCR-γ and 1.0-kb truncated TCR-β transcripts. In B-precursor ALL, 20 patients (69%) showed rearrangements of the TCR-δ gene. The frequency of TCR-δ gene rearrangement was higher than TCR-α (59%), γ (52%), or β (31%) genes. These findings suggest that TCR-α gene rearrangements may take place after rearrangements of the TCR-δ gene with concomitant deletion of rearranged TCR-δ genes in T cell differentiation. Among leukemic cells of B lineage, the TCR-δ gene is the earliest rearranging TCR gene, followed by TCR-γ and β gene rearrangements.

Introduction

Acute lymphoblastic leukemias (ALL)[1] are presumed to result from clonal expansion of normal developing cells (1–3). Molecular analyses of leukemic cells have offered important insights into our understanding of the developmental processes of lymphoid cells (4–11). We previously analyzed T cell receptor (TCR)-α gene configuration in T-ALL and B-precursor ALL using genomic joining region (Jα) probes covering the Jα region up to 85 kb 5′ to Cα (12, 13). All T-ALL with a mature phenotype (CD1⁻, CD3⁺) and 55% of B-precursor ALL showed rearrangements of the TCR-α gene. However, TCR-α gene rearrangements were not observed in the majority of CD3⁻ T-ALL or in CD1⁺, CD3⁺ T-ALL (13). An alternative TCR, in which a γ/δ heterodimer instead of α/β, is associated with the CD3 polypeptides, has been described (14–17). Thymocytes expressing TCR-γ/δ appear early in ontogeny and are replaced by cells expressing TCR-α/β. The subpopulation of cells expressing TCR-γ/δ is a minor one in the postnatal period (18, 19). Presently, little is known about the function of TCR-γ/δ, and it remains uncertain whether cells expressing TCR-γ/δ belong to the same lineage as T cells expressing TCR-α/β.

Recently, a novel constant region (C) gene was found in the TCR-α gene locus 75 kb, 5′ to Cα in mice (20). This gene is believed to encode the TCR-δ chain (21–24). Human cDNA clones analogous to this murine TCR-δ gene have also been recently isolated (25, 26). The human Cδ has been located between the TCR-α variable (V) region and the TCR-α J region, ~ 90 kb 5′ to Cα. The human TCR-δ region contains at least two defined Jδ loci (25).

In this study, we have analyzed TCR-δ gene configuration in 19 T-ALL and 29 B-precursor ALL to delineate the relationship between TCR-δ and TCR-α gene rearrangements. Analysis of TCR-δ gene rearrangements used newly defined TCR-δ cDNA and genomic probes. Transcription of the TCR-δ gene was also examined in 6 T-ALL patients.

Methods

Cell samples. Mononuclear cells were obtained from bone marrow by Ficoll-Hypaque 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 (3). The diagnosis of T-ALL was based on the expression of the T cell-associated antigens CD1, CD2, CD3, CD4, CD5, CD7, and CD8. Based on CD1 and CD3 expression, T-ALL was divided into four groups (Table I); group I, CD1⁻ and CD3⁻ (Pts. 1–4); group II, CD1⁺ and CD3⁻ (Pts. 5–7); group III, CD1⁺ and CD3⁺ (Pts. 8–14); and group IV, CD1⁻ and CD3⁺ (Pts. 15–19). In all cell samples, TCR-β or γ gene rearrangements were detected using the Cβ probe (YT35 0.8-kb Eco RV–Bg III cDNA fragment) (27) or the Jγ probe (0.7-kb Hind III–Eco RI fragment provided by Dr. T. H. Rabbitts) (28) as previously reported (10, 11).

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 (29). IgH gene rearrangements were observed in all B-precursor ALL samples using the Cμ probe (1.3-kb Eco RI fragment provided by Dr. T. H. Rabbitts) (30) as previously described (3). Results of TCR-γ and β gene rearrangements previously reported in part (10, 11) are shown in Table IV.

Southern blot hybridization. High-molecular weight DNA was extracted from mononuclear cells and digested with restriction endonu-

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1. *Abbreviations used in this paper:* C, constant region; J, joining region; TCR, T cell receptor; UT, untranslated region.

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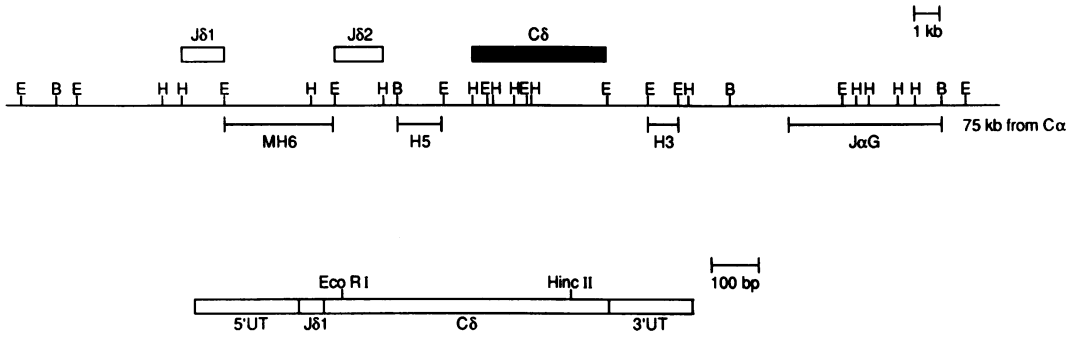

Figure 1. (*Top*) A restriction map of the TCR-δ gene and the genomic probes used in this study. E, H, and B denote Eco RI, Hind III, and Bam HI restriction sites, respectively. (*Bottom*) The cDNA probe (cTH2) used in this study.

cleases. The digested DNA was electrophoresed through 0.8% agarose gels and transferred to nylon membranes. Blots were hybridized to probes labeled with ^{32}P by the random primer method (31). The TCR-α gene probes used in this study included six genomic Jα probes, JαB, C, D, E (2.3-, 4.7-, 2.0- and 4.0-kb Eco RI fragments, respectively), JαF (1.5-kb Hind III fragment), and JαG (6.7-kb Bam HI fragment). These probes were described previously (12, 13). The JαB is the closest to Cα and the JαG is the furthest upstream, ~ 80 kb, as shown in Fig. 1. The H3 probe (0.9-kb Eco RI genomic fragment) was also used to analyze the configuration of the most upstream Jα region. The TCR-δ cDNA probe (cTH2) used in this study included the untranslated (UT) region 5′ to Jδ1, Jδ1, Cδ, and the UT region 3′ to Cδ (Fig. 1). In cases where rearrangements were observed, blots were rehybridized with the Cδ probe (0.5-kb Eco RI–Hinc II fragment of cTH2). The MH6 (4.5-kb Eco RI genomic fragment) and the H5 (1.5-kb Xba I genomic fragment) probes were also used to detect rearrangements involving the Jδ1 and Jδ2 loci, respectively.

Northern blot hybridization. Cytoplasmic RNA was extracted after lysis with NP-40 in the presence of 10 mM vanadyl-ribonucleoside complex and removal of nuclei from mononuclear cells (32). 10 μg of RNA was denatured in formamide, electrophoresed in 1% agarose gels containing formaldehyde, and transferred to nylon membranes. Blots were hybridized and washed under high stringency conditions (0.1 × SSC and 0.2% SDS at 65°C) (33). The probes used in this study were as follows: TCR-γ, HGPO3 1.4 kb cDNA (34); TCR-δ, cTH2 cDNA (described above); TCR-β, Cβ (described above); and TCR-α, Cα 0.35 kb Sau 3a-Hind III fragment of cDNA pGA5 (35).

Results

Rearrangement of the TCR-α gene in T-ALL. Results of TCR-α gene rearrangement observed using the JαB, JαC, JαD, JαE, JαF, and JαG probes were reported previously (12, 13), and are summarized in Table I. One (Pt. 4) of four group I, one

*Table I. TCR-δ and α Gene Rearrangement in 19 Patients with T-ALL**

					TCR-δ					
		Surface markers§				cDNA			Proposed Jδ gene usage	α-δ gene configuration
Patient	Group‡	CD4	CD8	TCR-α‖	MH6	5′UT	Cδ¶	H5		
1		−	−	G	R/D	R/D	G	G/R	δ2/δ1**	δ/δ
2	I	+	30	G	R/R‡‡	R‡‡/D	R/G	G	δ1/δ1**	δ/δ
3		−	−	G	R/R	D	G	G	δ1/δ1	δ/δ
4		+	+	R/R	D	D	D	D	D	α/α
5		+	42	G	R/D	D	R/G	G/R	δ1/δ2	δ/δ
6	II	+	+	G	R/R	D	G	G	δ1/δ1	δ/δ
7		−	+	R/G	R/D	D	R/D	G	δ1/D	δ/α
8		+	−	G	R/R	D	G	G	δ1/δ1	δ/δ
9		−	20	R/G	R/D	D	G	G	δ1/D	δ/α
10		40	40	G	R/D	D	G	G/R	δ1/δ2	δ/δ
11	III	19	−	R/R	D	D	D	D	D	α/α
12		10	+	R/G	R‡‡/D	D	R/D	G	δ1/D	δ/α
13		29	+	G	R/R	D	G	G	δ1/δ1	δ/δ
14		+	+	G	R/R	R/D	G	G	δ1/δ1**	δ/δ
15		−	−	R/R	D	D	D	D	D	α/α
16		27	−	R/G	R/D	R/D	G	G	δ1**/D	δ/α
17	IV	42	45	R/R	D	D	D	D	D	α/α
18		−	−	R/R	D	D	D	D	D	α/α
19		+	−	R/R	D	D	D	D	D	α/α

* G denotes germline, R, rearranged, and D, deleted. ‡ I: CD1⁻, CD3⁻; II: CD1⁺, CD3⁻; III: CD1⁺, CD3⁺; IV: CD1⁻, CD3⁺. § A minus sign denotes < 10% positive cells, and a plus sign > 50% positive cells; numbers are specific percentages of positive cells. ‖ Results using the JαB, C, D, E, F, and G probes (13). ¶ Results after Bam HI and Hind III digestion. ** Rearrangements of the region upstream to the Jδ1 gene. ‡‡ Identified by the H3 probe.

(Pt. 7) of three group II, three (Pts. 9, 11, and 12) of seven group III, and all five (Pts. 15–19) group IV patients had rearrangements of the TCR-α gene. In four of these patients (Pts. 7, 9, 12, and 16), germline configuration was retained on the other allele.

TCR-δ gene rearrangement in T-ALL. DNA samples were digested with Eco RI, Bam HI, or Hind III and hybridized with the MH6, H5, or cDNA probes. Rearrangement patterns and representative Southern blots are shown in Table I and Figs. 2–4. The MH6 probe revealed a 14-kb Bam HI band and two Hind III bands (5 and 2.6 kb) (Fig. 2). The 14-kb Bam HI band and the 5-kb Hind III band contained the Jδ1 gene. Hybridization with the H5 probe yielded a 4-kb Eco RI band containing the Jδ2 gene (Fig. 3). The Eco RI fragment identified by the MH6 probe, and Bam HI and Hind III fragments identified by the H5 probe could not be used to demonstrate rearrangements, as these fragments did not contain the Jδ1 or Jδ2 genes (Fig. 1).

All 13 patients with germline configuration of the TCR-α gene on at least one allele showed rearrangements of the TCR-δ gene using the MH6 probe and either Bam HI or Hind III digestion (Fig. 2). Bi-allelic Jδ1 rearrangements were observed in 6 (Pts. 2, 3, 6, 8, 13, and 14) of these 13 patients. The remaining seven patients (Pts. 1, 5, 7, 9, 10, 12, and 16) showed single allelic Jδ1 rearrangements without retention of germline configuration of the TCR-δ gene. When these seven patients were examined for Jδ2 rearrangement using the H5 probe, three patients (Pts. 1, 5, and 10) demonstrated single allelic rearrangements after Eco RI digestion (Fig. 3). These findings suggest that these three patients had rearrangements to Jδ1 on one allele and to Jδ2 on the other allele. In all six patients with TCR-α gene rearrangement on both alleles (Pts. 4, 11, 15, and 17–19), the MH6 and H5 Jδ bands were deleted.

The cDNA probe (cTH2) was used to analyze TCR-δ gene configuration in more detail. After Eco RI digestion, a 6-kb band corresponding to the 5′UT and Jδ1, and three bands (3, 1.6, and 1.4 kb) corresponding to Cδ and the 3′UT were observed in germline DNA (Figs. 1 and 4). Bam HI digests yielded two germline bands, a 14-kb band containing Jδ1 and Jδ2, and a 12-kb band containing all the Cδ sequences (Figs. 1 and 4). The sequences of the cDNA probe encoding the Jδ1 gene were too small to hybridize to the Jδ1 gene under the high-stringency washing conditions used in this study. In cases of rearrangements to the Jδ1 gene, therefore, Jδ1 bands appeared to be deleted because the 5′UT region recognized by the cDNA probe (Fig. 1) is deleted in these types of rearrangements.

After Eco RI or Bam HI digestion, nine patients with rearrangements previously identified by the MH6 probe showed deletion of Jδ1 bands on both alleles using the cDNA probe,

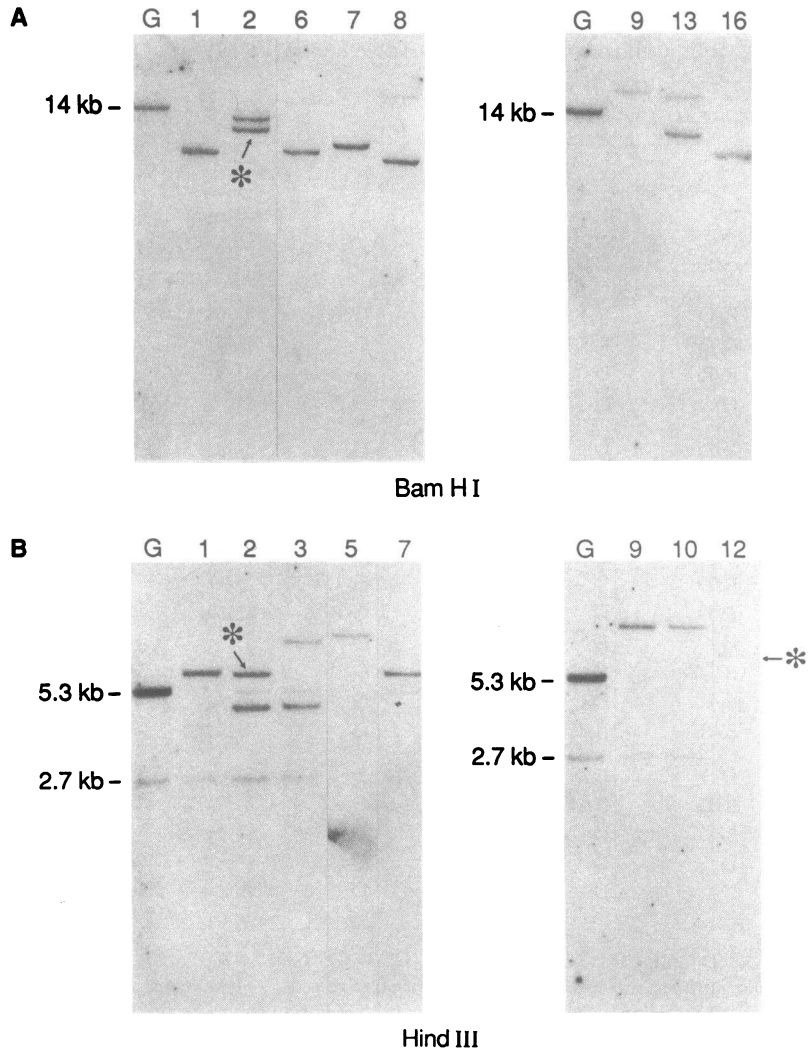

Figure 2. Representative rearrangement patterns of the TCR-δ gene in T-ALL. The patient numbers are noted above each lane. Lane *G* shows the germline control. (*A*) Bam HI and (*B*) Hind III digests were probed with the MH6 probe. Asterisks indicate the fragments also identified by the H3 probe. (*A*) Bam HI digests yielded a 14-kb band in germline DNA. (*B*) Hind III digests yielded 5.3- and 2.7-kb bands in germline DNA.

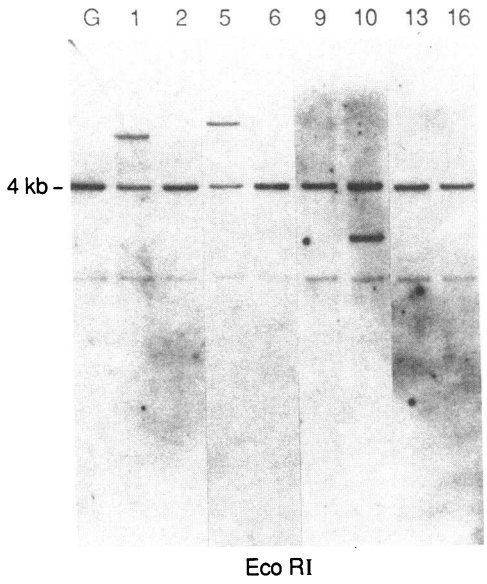

Figure 3. Rearrangement patterns of the TCR-δ gene in T-ALL after Eco RI digestion and hybridization with the H5 probe. The H5 probe demonstrated a 4-kb band in germline DNA. Faint bands observed below the H5 bands are due to cross-hybridization.

suggesting rearrangements to the Jδ1 gene. In four patients (Pts. 1, 2, 14, and 16), rearranged bands not identified by the Cδ probe (data not shown) were observed without germline Jδ1 bands. These rearranged bands were also identified by the cDNA probe after Hind III digestion. As expected, rearranged Bam HI and Hind III bands were visualized with the MH6 probe. Usage of Jδ genes is summarized in Table I.

In three patients (Pts. 5, 7, and 12), after Bam HI digestion the cDNA probe demonstrated rearranged fragments identified by the Cδ probe despite germline configuration of Eco RI Cδ fragments (Table I). Similar results were obtained by Hind III digestion; 6.3-kb Hind III Cδ fragments corresponding to the most 3′ region of Cδ also showed rearrangements (data not shown). Hybridization with the H3 probe demonstrated germline Eco RI bands, but after Bam HI and Hind III digestion, rearranged bands were observed in these three patients (data not shown). Because sizes of rearranged H3 bands were different from those identified by the Cδ probe, there was discontinuity between H3 and Cδ genes. Furthermore, the rearranged Bam HI and Hind III Jδ1 bands in Pt. 2 and the rearranged Hind III Jδ1 band in Pt. 12 were identified by the H3 probe suggesting recombination between Jδ1 and the region identified by the H3 probe.

TCR gene transcription in T-ALL. Results of TCR-γ, β, and α gene transcription reported previously (13) are summarized in Table II. TCR-δ gene transcription was analyzed in six patients using the cDNA probe. Northern blots are shown in Fig. 5. The leukemic T cell line, PEER, with TCR-γ/δ on the cell surface (17) expressed four sizes of transcripts, 2.2, 2.0, 1.5, and 1.3 kb. Of these, 2.2- and 1.5-kb transcripts were predominantly expressed and the 2.0- and 1.3-kb transcripts were visible in a longer exposure of the experiment presented in Fig. 5. 2.2- and 1.5-kb transcripts are composed of full-length mRNA containing V regions; on the other hand, 2.0- and 1.3-kb transcripts are immature and lack V region transcription (25). The size differences between 2.2- and 1.5-kb transcripts and between 2.0- and 1.3-kb transcripts are most likely due to differences in polyadenylation sites (25). One patient (Pt. 1), who only expressed the CD7 antigen and therefore presumably derived from immature T lineage cells, did not express the TCR-δ gene. In this patient, neither TCR-α nor full-length

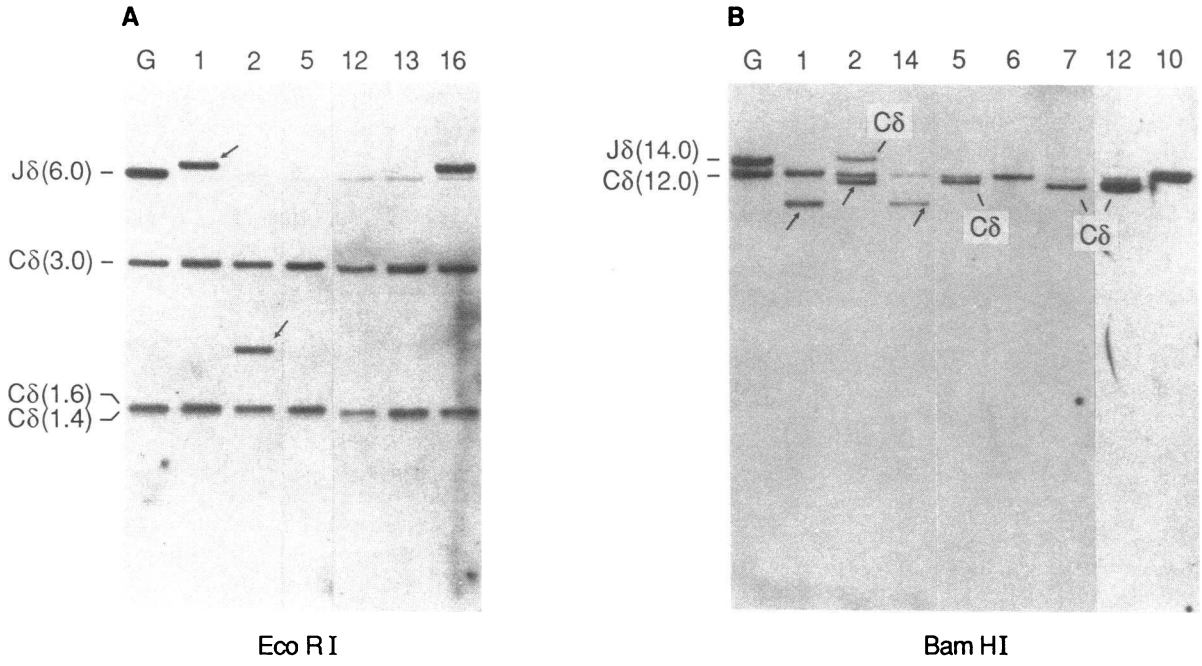

Figure 4. Representative rearrangement patterns of the TCR-δ gene in T-ALL. (*A*) Eco RI and (*B*) Bam HI digests were probed with the cDNA probe (cTH2). Rearranged bands are indicated by arrows on the blots. (*A*) Eco RI digests yielded a 6.0-kb band corresponding to the 5′UT and Jδ1 and 3 bands, 3.0, 1.6, and 1.4 kb corresponding to the Cδ. (*B*) Bam HI digests yielded a 14-kb band corresponding to the 5′UT and Jδ1 and a 12.0-kb band corresponding to the Cδ. Rearranged bands corresponding to Cδ are indicated by bars on the blot.

Table II. Transcription of TCR Genes in 11 Patients with T-ALL

Patient	Group*	Rearrangement δ, α	Transcription‡ δ 2.2	δ 2.0	δ 1.5	δ 1.3	γ 1.7	β 1.3	β 1.0	α 1.6	α 1.3
1		δ/δ	−	−	−	−	+	−	+	−	−
2	I	δ/δ	+(wk)	+	+(wk)	+	+	+	−	−	−
4		α/α						+	+	−	−
9		δ/α	+(wk)	+	+(wk)	+	+	+	+	+	−
10	III	δ/δ	+	+(wk)	+	+(wk)	+	−	+	+(wk)	+(wk)
11		α/α					−	+	+	+	−
12		δ/α	−	+(wk)	+(wk)	+	+	+	+	+(wk)	+(wk)
16		δ/α	+(wk)	+	+(wk)	+	+	+	−	+	−
17	IV	α/α					+	+	+	+	−
18		α/α					+	+	+	+	+
19		α/α					−	+	−	+	−

* I: CD1$^-$, CD3$^-$; III: CD1$^+$, CD3$^+$; IV: CD1$^-$, CD3$^+$. ‡ Size in kilobases. + denotes readily detectable expression; +(wk), weak expression; −, expression was not detected.

1.3-kb TCR-β transcripts were observed, but the TCR-γ gene was expressed. The remaining five patients (Pts. 2, 9, 10, 12, and 16) expressed the TCR-δ gene along with TCR-γ transcripts. The transcriptional intensity patterns in four patients (Pts. 2, 9, 12, and 16) were different from those in PEER. Levels of 2.2- and 1.5-kb transcripts were low compared with 2.0- and 1.3-kb transcripts. Only one patient (Pt. 10) strongly expressed 2.2- and 1.5-kb TCR-δ transcripts and showed a similar transcriptional intensity pattern to that observed with PEER cells. In this patient, TCR-γ and only 1.0-kb truncated TCR-β transcripts were detected. In addition, weak expression of the TCR-α gene was demonstrated. It is likely that these TCR-α transcripts may be derived from the TCR-α gene in germline configuration.

TCR-α gene rearrangement in B-precursor ALL. Results of TCR-α gene configuration in B-precursor ALL obtained using the Jα probes will be published elsewhere (36a). 17 patients showed rearrangements of the TCR-α gene. In five patients (Pts. 44–48), TCR-α gene rearrangements were bi-allelic and in 12 patients (Pts. 32–43), the rearrangement was on a single allele (Table III).

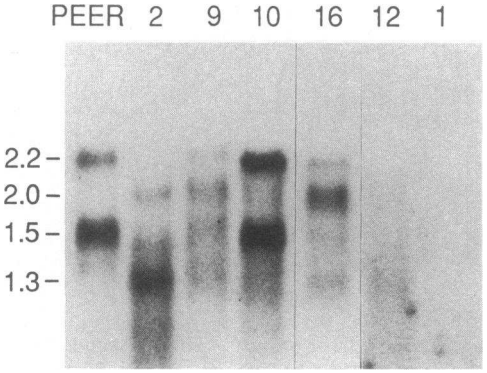

Figure 5. Transcription of the TCR-δ gene. Sizes of transcripts are indicated on the left side of the blot.

TCR-δ gene rearrangement in B-precursor ALL. Results in 29 B-precursor ALL obtained using the cDNA and MH6 probes are summarized in Tables III and IV. Representative Southern blots are shown in Fig. 6. TCR-δ gene rearrangement was observed in 20 patients (Pts. 23–31 and 33–43). 11 (Pts. 33–43) of these 20 patients also had rearrangements of the TCR-α gene on the other allele. In five patients (Pts. 44–48), the TCR-δ locus was deleted on both alleles and TCR-α gene was rearranged. In contrast to findings in the majority of T-ALL, all B-precursor ALL with TCR-δ gene rearrangement showed an unusual rearrangement pattern. Rearranged Jδ1 bands were observed when Eco RI or Bam HI digests were probed with the cDNA probe. This indicated that intervening sequences between Dδ and Jδ1 genes were preserved in these rearrangements and suggests that unlike the case with Ig genes, D to J rearrangement may not be the first event in TCR-δ rearrangement.

As expected, rearranged Bam HI bands were also identified by the MH6 probe (data not shown). As shown in Fig. 6, all rearrangements, except for those observed in patients with three rearrangements, fell into only three groups. The H5 probe failed to demonstrate rearrangements in any patients with B-precursor ALL (data not shown). Two patients (Pts. 25 and 27) with germline configuration of the TCR-α gene on both alleles had only a single MH6 rearranged band. It is possible that rearranged bands on both alleles were the same size and overlaid each other, preventing discrimination. Based on the configuration of the TCR-δ and α genes, 29 patients with B-precursor ALL were divided into five groups, as shown in Tables III and IV. Only three patients (group A) showed germline configuration of both the TCR-δ and α genes. Nine patients (group B) had rearrangements of the TCR-δ gene with germline configuration of the TCR-α gene. In one patient (group C), TCR-α gene rearrangement (JαG) was observed with a single germline allele of the TCR-δ gene. The remaining 16 patients showed rearrangements of the TCR-α gene. Of these, 11 patients (group D) also showed rearrangements of the

Table III. TCR-α and δ Gene Rearrangement in 29 Patients with B-precursor ALL

Pt.	Group*	TCR-α‡	TCR-δ MH6	TCR-δ cDNA (5'UT)	α-δ gene configuration
20	A	G§	G	G	G
21		G	G	G	G
22		G	G	G	G
23	B	G	R/G	R/G	δ/G
24		G	R/G	R/G	δ/G
25		G	R/D	R/D	δ/δ
26		G	R/R	R/R	δ/δ
27		G	R/D	R/D	δ/δ
28		G	R/R/R	R/R/R	δ/δ/δ
29		G	ND	R/G	δ/G
30		G	R/R	R/R	δ/δ
31		G	R/R	R/R	δ/δ
32	C	R/G	D/G	D/G	α/G
33	D	G/R	ND	R/D	δ/α
34		G/R	R/D	R/D	δ/α
35		G/R	R/D	R/D	δ/α
36		G/R	R/D	R/D	δ/α
37		G/R	R/D	R/D	δ/α
38		G/R	R/D	R/D	δ/α
39		G/R	R/D	R/D	δ/α
40		G/R	R/D	R/D	δ/α
41		G/R	R/D	R/D	δ/α
42		G/R	R/D	R/D	δ/α
43		G/R	R/D	R/D	δ/α
44	E	R	D	D	α/α
45		R	D	D	α/α
46		R	D	D	α/α
47		R	D	D	α/α
48		R	D	D	α/α

* See text for description of group designations.
‡ Results using the JαB, C, D, E, F, and G probes (36a).
§ G denotes germline, R, rearranged, and D, deleted.

TCR-δ gene and in 5 patients (group E) TCR-δ was deleted on both alleles.

Discussion

In this study, we have analyzed TCR-δ gene configuration in T-ALL and B-precursor ALL using a newly isolated human TCR-δ cDNA probe and various genomic probes. Among 19 T-ALL patients, 9 patients demonstrated rearrangements of the TCR-δ gene on both alleles and 4 patients were rearranged on a single allele with rearrangements of the TCR-α gene on the other allele. The remaining six patients had biallelic TCR-α gene rearrangements and the TCR-δ gene was deleted. Transcription of the TCR-δ gene was detected in five of six patients analyzed. B-precursor ALL also showed a high frequency of TCR-δ gene rearrangements. 20 of 29 B-precursor ALL patients had rearrangements of the TCR-δ gene and 11 of these 20 patients had TCR-α gene rearrangements on the other allele. Of the remaining nine patients, bi-allelic TCR-α gene rearrangements were observed in five patients and one patient had a single allelic TCR-α gene rearrangement with germline configuration of the TCR-δ and α genes. Only three patients did not show rearrangement of the TCR-δ or α genes.

Based on the expression of the CD1 and CD3 antigens, the patients with T-ALL studied here were divided into 4 subgroups; group I, $CD1^-$ and $CD3^-$; group II, $CD1^+$ and $CD3^-$; group III, $CD1^+$ and $CD3^+$; and group IV, $CD1^-$ and $CD3^+$. These groups are postulated to represent progressive stages of T cell differentiation. Three of four group I patients (75%) and two of three group II patients (67%) had bi-allelic rearrangements of the TCR-δ gene. One of the group I patients had bi-allelic TCR-α gene rearrangements, and one of the group II patients showed TCR-δ and TCR-α gene rearrangements on each allele. Among the $CD3^+$ T-ALL patients, four of seven group III patients (71%) showed bi-allelic rearrangements of the TCR-δ gene and two patients showed TCR-δ rearrange-

Table IV. Summary of TCR Gene Rearrangement in 29 Patients with B-precursor ALL

Pt.	Group	T cell receptor genes δ	α	γ	β
20	A	G*	G	G	G
21		G	G	G	G
22		G	G	G	G
23	B	R/G	G	G	G
24		R/G	G	G	G
25		R	G	G	G
26		R	G	G	G
27		R	G	R/G	G
28		R/R/R	G	R/G	G
29		R/G	G	R/G	G
30		R	G	R	G
31		R	G	R/G	G
32	C	D/G	R/G	G	G
33	D	R/D	G/R	G	G
34		R/D	G/R	G	G
35		R/D	G/R	G	G
36		R/D	G/R	G	G
37		R/D	G/R	G	G
38		R/D	G/R	R/G	G
39		R/D	G/R	R/G	G
40		R/D	G/R	R	R/G
41		R/D	G/R	R	R
42		R/D	G/R	R	R/D
43		R/D	G/R	R	R/G
44	E	D	R	G	R/D
45		D	R	R	R
46		D	R	R	R/G
47		D	R	R	R/G
48		D	R	R	R/G

* G denotes germline, R, rearranged, and D, deleted.

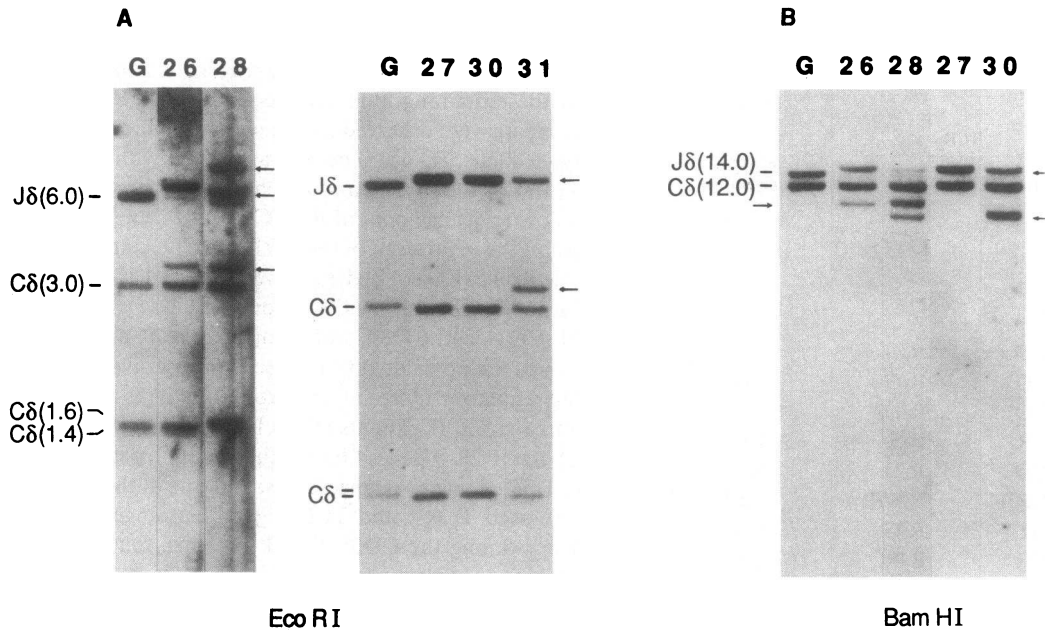

Figure 6. Representative rearrangement patterns of the TCR-δ gene in B-precursor ALL. (*A*) Eco RI and (*B*) Bam HI digests were probed with the cDNA probe (cTH2). Rearranged bands are indicated by arrows on the blots.

ment on one allele and TCR-α gene rearrangement on the other allele. Bi-allelic rearrangements of the TCR-α gene were observed in the remaining patient. In contrast to results with group III, all group IV patients had TCR-α gene rearrangement and only one patient showed TCR-δ gene rearrangement in addition to TCR-α gene rearrangement. Based on these findings, it seems that at early stages of differentiation, TCR-δ gene rearrangement may initially occur and during further differentiation, rearrangements of the TCR-α gene may take place with concomitant deletion of a preexisting VDJ Cδ complex. It is possible that successful rearrangement of the TCR-δ gene together with functional TCR-γ gene rearrangement may proceed directly to differentiate into the T cell subset expressing TCR-γ/δ on the cell surface. If, however, TCR-δ or TCR-γ rearrangements prove nonfunctional, this may result in further rearrangement of the TCR-α gene locus and these cells would differentiate into T cells expressing TCR-α/β. Analogous findings have been observed with Ig genes where functional rearrangements of the Ig-κ gene may inhibit activation of the Ig-λ gene locus; failure of successful rearrangement of the Ig-κ gene initiates Ig-λ gene rearrangement with deletion of the Ig-κ gene locus (36). Alternatively, these two sets of T cells may derive from separate lineages.

Interestingly, most group III patients ($CD1^+$ and $CD3^+$) showed rearrangement of the TCR-δ gene with germline configuration of the TCR-α gene. It has been thought that expression of the CD3 complex on the cell surface may require the presence of the TCR-α/β heterodimer (37–40). If one assumes that the TCR-γ/δ heterodimer may substitute for TCR-α/β in CD3 antigen expression, the $CD3^+$ cells with bi-allelic TCR-δ gene rearrangement observed in this study should have the TCR-γ/δ in association with the CD3 complex. The majority of freshly isolated $CD3^+$ double negative ($CD4^-$ and $CD8^-$) thymocytes expressed the CD1 antigen and after culture with IL2, CD1 disappeared from the surface of cells expressing TCR-γ/δ (15). It is, therefore, likely that a large proportion of cells with TCR-γ/δ may express CD1 in vivo. In contrast to group III patients, all five group IV patients had rearrangements of the TCR-α gene. It would be interesting to know whether those γ/δ-bearing cells, belonging to group III, differentiate into group IV cells with replacement of TCR-γ/δ by TCR-α/β.

As shown in Table I, 15 of the 22 total rearrangements of the TCR-δ gene in T-ALL used the Jδ1 gene. In contrast, use of the Jδ2 gene was observed only in three rearrangements. The remaining four rearrangements in T-ALL and all rearrangements observed in B-precursor ALL were incompatible with ordinary DJδ or VDJδ rearrangements. In these rearrangements, intervening sequences between Dδ and Jδ1 genes were preserved, suggesting that VDδ rearrangements might in some cases precede rearrangement of Dδ to Jδ. Sequence data (26) suggest the presence of a D gene at the 5′ terminus of the Jδ1 locus (Hind III-Eco RI fragment) and that no Jδ genes other than the Jδ1 gene encoded by the cDNA probe exist within this locus. It therefore seems that rearranged bands identified by the cDNA probe did not result from ordinary DJδ1 or VDJδ1 recombinations. The likely explanation of this phenomenon is a VDδ rearrangement with preservation of the intervening sequences between Dδ and Jδ1 genes. Alternatively, some unknown mechanism regulating the assembly of the TCR-δ gene might have resulted in these rearrangements. This type of rearrangement has been demonstrated for the TCR-δ gene in fetal mouse thymocytes (41), and seems to occur first in these cells at least as frequently as D to J rearrangements.

Four patients with T-ALL (Pts. 2, 5, 7, and 12) showed curious patterns of rearrangement which were difficult to explain by ordinary recombinational models. In these patients, discontinuity between the rearranged Cδ and the region just 5′ to H3 was observed. In Pt. 2, recombination between Jδ1 and the region just 5′ to H3 was demonstrated with retention of germline configuration of the Jδ2 and Cδ genes. Patient 12 also showed recombination between these two loci after Hind III digestion. Although precise characterization of the other recombination sites was not completed, these findings suggest that an inversion of the locus that included the Jδ and Cδ genes had occurred. Recently, repetitive elements located around this region analogous to switch regions in the IgH constant region have been described (42). These elements remained in

germline configuration in T cells expressing TCR-γ/δ and were deleted from T cells expressing TCR-α/β. Rearrangement of these elements has only been observed in polyclonal thymocytes, including putative cells showing transient characteristics between immature T cells, which express TCR-γ/δ and mature T cells, which express TCR-α/β (42). Although the location of these repetitive elements in the genome is unknown, it is possible that leukemic cells from these patients with rearrangements of the region just 5′ to H3 may be arrested at a normally transient stage between stages expressing TCR-γ/δ and cells expressing TCR-α/β, and recombinational events observed in these patients may reflect a transition between these two stages. We are currently attempting to delineate the recombinational event observed here by sequencing the potential recombination sites. Alternatively, since the Jα region, and probably the TCR-δ gene locus are frequently involved in translocations in T cell neoplasia (43–45), chromosomal abnormalities involving chromosome 14 may have caused these abnormalities at this gene locus. Indeed, Pt. 5 had a translocation of t(10;14) (q 24; q 11). Recent data from Isobe et al. support this possibility (46).

To assess expression of the TCR-δ gene, Northern blot analyses were performed using the cDNA probe. There were four sizes of TCR-δ transcripts; these were 2.2, 2.0, 1.5, and 1.3 kb. The 2.2- and 1.5-kb species have been shown to be functional transcripts containing VDJ sequences and these were predominantly expressed in the leukemic T cell line PEER expressing TCR-γ/δ on the surface (25). A similar pattern of TCR-δ transcripts was observed in Pt. 10 with bi-allelic TCR-δ rearrangement. In this patient, TCR-γ transcripts were also detected but 1.3-kb, full-length TCR-β transcripts were not present (Table II). It is likely that the cells from Pt. 10 have TCR-γ/δ in association with the cell surface CD3 complex. Conversely, 2.0- and/or 1.3-kb transcripts lacking V regions (25) were abundant, compared with 2.2- and 1.5-kb transcripts in four patients (Pts. 2, 9, 12, and 16). Of these, the three $CD3^+$ T-ALL (Pts. 9, 12, and 16) also had full-length, 1.6-kb TCR-α transcripts and full-length, 1.3-kb TCR-β transcripts, suggesting expression of the TCR-α/β complex on the cell surface. One patient (Pt. 1), presumably representing the most immature T cells, failed to express TCR-δ transcripts, despite expression of TCR-γ and 1.0-kb immature TCR-β transcripts. Although surprising, it is possible that some TCR-δ transcription occurred earlier, to facilitate TCR-δ rearrangement. This TCR-δ transcription may have been abortive and the TCR-γ transcription now observed reflects a subsequent step in development. Thus transcription of the fully rearranged TCR-δ gene may be preceded by transcription of rearranged TCR-γ and TCR-δ transcripts might be a critical event for TCR-γ/δ expression on the cell surface in a situation analogous to the relationship between the TCR-β and α genes.

Among 29 patients with B-precursor ALL, 20 patients (69%) showed rearrangements of the TCR-δ gene. The frequency of TCR-δ gene rearrangement was higher than TCR-α (59%), γ (52%), and β (31%) genes. Nine patients showed TCR-δ gene rearrangement without rearrangements of the TCR-γ or β genes and TCR-β gene rearrangement accompanied TCR-γ gene rearrangement in all but one patient. Although, as discussed above, the nature of these rearrangements of TCR-δ is still obscure, it appears that at least in leukemic cells of B lineage, TCR-δ gene rearrangement is the earliest event among rearrangements of the TCR genes. It is also possible that rearrangements of the TCR-δ and TCR-γ genes may be followed by rearrangements of the TCR-α and TCR-β genes, respectively. TCR-α and δ rearrangement may in turn be independently regulated by some protein products of rearranged TCR-δ and γ genes. To conclude whether the hierarchy derived from this study of B lineage cells reflects the order of TCR gene rearrangements in T lineage cells, it will be necessary to analyze a greater number of immature cell samples.

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