

Comparison of T Cell Receptor α , β , and γ Gene Rearrangement and Expression in T Cell Acute Lymphoblastic Leukemia

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Abstract

We have analyzed the configuration of the T cell receptor (TCR) α gene using newly developed genomic joining region ($J\alpha$) probes, which cover ~ 80 kb of the $J\alpha$ region upstream from the constant region in 19 patients with T cell acute lymphoblastic leukemia (T-ALL) and in three CD3⁻ leukemic T cell lines (HSB2, CEM, and MOLT4). In parallel, transcription of the TCR- α , β , and γ genes was examined in 11 of these patients and in the T cell lines. All T-ALL and the three T cell lines exhibited both TCR- γ and β gene rearrangements. 8 of 10 T-ALL and all T cell lines expressed TCR- γ transcripts. All samples tested expressed both TCR- β and CD3- γ transcripts. TCR α transcripts were only observed in CD3⁺ T-ALL but not in CD3⁻ T-ALL or the CD3⁻ cell lines. Among the CD3⁺ T-ALL, eight had TCR- α gene rearrangements. In addition, TCR- α gene rearrangements were detected in one CD3⁻ T-ALL and all three T cell lines. These leukemic cells may represent a transient stage between rearrangement and expression and provide an opportunity for analyzing the mechanism regulating the expression of the TCR- α gene.

Introduction

Acute lymphoblastic leukemias (ALL)¹ are presumed to result from clonal expansion of particular stages of developing lymphoid cells and provide unique opportunities to analyze molecular events associated with lymphoid cell differentiation at a clonal level. Molecular analyses of these cells have allowed the description and characterization of gene rearrangement and transcription providing models for the developmental hierarchy of immunoglobulin and T cell receptor (TCR) gene activation (1–10).

The T cell antigen receptor or Ti heterodimer, consists of the TCR- α and β chains, which are encoded by functionally rearranged TCR- α and TCR- β genes, respectively (11–14). In

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

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close association with Ti are at least three polypeptides (CD3- γ , CD3- δ , and CD3- ϵ). Together, these proteins form the core of the antigen-specific TCR, the CD3- α/β complex (15, 16). Recently, the protein product of a third rearranging gene TCR- γ has been identified (17, 18). This protein can be expressed in association with the CD3 complex on the cell surface together with TCR- δ in the absence of the α/β heterodimer (19, 20). However, it is still uncertain whether cells expressing the TCR- γ and δ polypeptides belong to the same lineage as T cells expressing the CD3- α/β complex.

Developmental studies in the murine fetus suggest that TCR- γ gene rearrangement and expression occur first, followed by TCR- β gene rearrangement and expression. Thereafter, during further differentiation, transcription of the TCR- γ gene decreases, while TCR- α transcription increases (21–26). Moreover, as shown in T-ALL cells, the CD3 proteins can combine with TCR- β protein in the cytoplasm of CD3⁻ immature cells, whereas TCR- α transcripts and protein have only been observed in CD3⁺ cells (27–30). Expression of the TCR- α gene may thus serve as the critical regulatory event in the formation of the CD3-Ti complex at the cell surface (26). In view of this, it has been postulated that TCR- α gene rearrangement may follow TCR- β gene rearrangement, analogous to the relationship between immunoglobulin heavy and light chain genes in B cells (31).

The TCR- α gene consists of more than 60 joining (J) gene segments in a region of 90 kb, located 5' to a single constant (C) gene (Champagne E., and M. Minden, unpublished observations). Using genomic $J\alpha$ probes that cover 45 kb of the $J\alpha$ region upstream from $C\alpha$, we failed to detect rearrangement of the TCR- α gene even in CD3⁺ T-ALL cells (32). We suggested that TCR- α gene rearrangement may therefore frequently occur in a $J\alpha$ region > 45 kb from $C\alpha$. To resolve these issues, newly developed $J\alpha$ probes capable of detecting rearrangements of $J\alpha$ up to 80 kb from $C\alpha$ were used. In addition, genomic or cDNA probes for the TCR- α , β , γ , and CD3- γ genes were employed to delineate the hierarchy of TCR gene activation in T-ALL and leukemic T cell lines arrested at different stages of T cell development.

Methods

Cell samples. Mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation of bone marrow samples obtained from 19 children with untreated T-ALL. The samples evaluated contained $> 90\%$ malignant cells. The diagnosis of T-ALL was established by reactivity with T cell-associated monoclonal antibodies, CD1, CD2, CD3, CD4, CD5, CD7, and CD8 (3). Based on the expression of the CD1 and CD3 antigens, these patients were divided into four subgroups as shown in Table I; stage I, CD1⁻, CD3⁻ (patients 1 through 4); stage II, CD1⁺, CD3⁻ (patients 5 through 7); stage III, CD1⁺, CD3⁺ (patients 8 through 14); and stage IV, CD1⁻, CD3⁺ (patients 15 through 19). Three estab-

Table I. Surface Antigen Expression in 19 Patients with T Cell ALL and Leukemic T Cell Lines

Patient No.	Stage*	Surface markers†					
		CD7	CD3	CD1	CD2	CD4	CD8
1		+	-	-	-	-	-
2	I	ND	-	-	20	+	30
3		+	-	-	+	-	-
4		ND	-	-	+	+	+
5		ND	-	+	+	+	42
6	II	ND	-	+	+	+	+
7		ND	-	30	+	-	+
8		+	+	20	-	+	-
9	III	ND	18	18	+	-	20
10		ND	+	+	+	40	40
11		ND	+	44	+	19	-
12		ND	15	+	+	10	+
13		ND	+	30	+	29	+
14		ND	30	+	+	+	+
15		+	+	-	+	-	-
16	IV	ND	+	-	-	27	-
17		ND	33	-	+	42	45
18		ND	+	-	+	-	-
19		ND	+	-	+	+	-
HSB2		+	-	-	+	-	-
CEM		+	-	-	+	+	+
MOLT4		+	-	-	-	+	-

* 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.

lished leukemic T cell lines (HSB2, CEM, and MOLT4) were also analyzed. In all cell samples, TCR- β and γ gene rearrangements were detected using the C β probe (YT35 0.8 kb Eco RV-Bgl II cDNA fragment) (11) and the J γ probe (0.7 kb Hind III-Eco RI fragment provided by T. H. Rabbitts) (33) as previously reported (5, 7).

Southern blot hybridization. High molecular weight DNA was prepared from mononuclear cells and digested with appropriate restriction endonucleases. The digested DNA was electrophoresed through 0.6–0.9% agarose slab gels, and transferred to nylon membranes. Blots were hybridized to probes labeled to $\sim 2 \times 10^9$ cpm/ μ g with ³²P by the random primer method (34). The TCR- α gene probes used in this study included five genomic J α probes. A diagram of TCR- α gene J region and these probes is shown in Fig. 1. The J α C and J α D probes (4.7- and 2.0-kb fragments, respectively) were described previously (35). Probes J α E, J α F, and J α G (4.0-, 1.5-, and 6.7-kb fragments, respectively) were isolated in the laboratory of M. Minden. The description of the isolation of these clones and a detailed map will be published elsewhere (Champagne E., and M. Minden, unpublished observations). Each DNA fragment contains several J α segments. As shown in Fig. 1, the J α C probe is the closest to C α and the J α G probe is the furthest upstream, ~ 80 kb. To investigate TCR- α gene rearrangement, all samples were digested with either Bam HI or Hind III, and to detect allelic deletion clearly, blots were hybridized repeatedly with probes in various combinations. The intensities of hybridized frag-

ments were compared to germline controls by densitometry. C μ or C κ fragments were also used as internal controls to verify amounts of DNA loaded, because in these combinations, Bam HI digests with the C κ probe, and Hind III digests with the C μ probe, showed a germline configuration in all samples. In cases where rearranged J α bands were demonstrated, the rearranged band was rescreened by hybridization with single probes.

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 11 T-ALL samples and the three T cell lines, as described previously (36). 10 μ g of RNA was denatured in formamide, electrophoresed in 1% agarose containing formaldehyde, and transferred to nylon membranes. After hybridization, blots were washed in high stringency conditions (0.1 \times SSC and 0.2% SDS) (37). The probes used in this study were as follows: TCR- γ , HGP03 1.4 kb cDNA (38); C β (described above); C α , pGA-5 0.35 kb Sau 3a-Hind III cDNA fragment provided by J. Kappler (14); and CD3- γ , 0.6 kb cDNA (38).

Results

DNA analysis. All T-ALL patients had rearrangements of both the TCR- γ and β genes, as described previously (5, 7). The

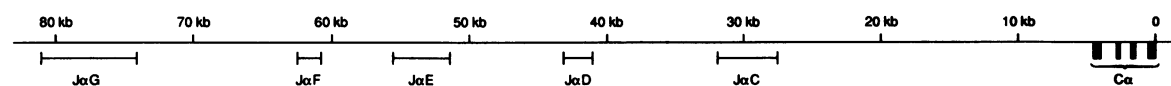


Figure 1. Diagram of the TCR- α gene and the genomic J α probes used in this study. Numbers indicate distance from the constant region.

Table II. Rearrangement and Expression of T Cell Receptor Genes in 19 Patients with T Cell ALL and Leukemic T Cell Lines

Patient No.	Stage	T cell receptor gene rearrangement*			T cell receptor gene transcription [‡]					
		γ	β	α	γ	β		α		CD3- γ
						1.3 kb	1.0 kb	1.6 kb	1.3 kb	
1	I	R/R	R/R	G	+ [§]	-	+	-	-	+
2		R/R	R/R	G	+ (wk)	+	-	-	-	+
3		R/R	R/D	G						
4		R/R	R/D	R/R		+	+	-	-	+
5	II	R/R	R/D	G						
6		R/R	R/D	G						
7		R/R	R/D	G						
8	III	R/R	R/R	G						
9		R/R	R/R	R/G	+	+	+	+	-	+
10		R/D	R/D	G	+	-	+	+ (wk)	+ (wk)	+
11		R/R	R/R	R/R	-	+	+	+	-	+
12		R/R	R/D	R/G	+	+	+	+ (wk)	+ (wk)	+
13		R/R	R/R	G						
14		R/R	R/R	G						
15	IV	R/R	R/R	R/R						
16		R/R	R/R	R/G	+	+	-	+	-	+
17		R/R	R/R	R/R	+	+	+	+ (wk)	-	+
18		R/R	R/R	R/R	+	+	+	+	+	+
19		R/D	R/G	R/R	-	+	-	+	-	+
HSB2		R/R	R/R	R/R	+	+	+	-	-	+
CEM		R/R	R/R	R/R	+ (wk)	+	-	-	-	+
MOLT4		R/R	R/R	R/R	+ (wk)	+	+	-	-	+

* R: denotes rearranged, G: germline and D: deleted. [‡] +: denotes normal expression, + (wk): weak expression, -: expression was not detected. [§] In addition to a 1.7-kb transcript, a 1.3-kb transcript was observed in this patient. ^{||} After Bam HI digestion, the rearranged band probably resulted from a polymorphic Bam HI restriction site in this patient. In addition, single allelic deletions corresponding to the J α D, J α E, and J α F probes were detected.

profiles of TCR- γ and β gene rearrangements are shown in Table II. A diagram of the TCR- α gene and the genomic J α probes used in this study are shown in Fig. 1. If a variable (V α) segment recombines with a J α segment, intervening J α sequences located upstream of the recombination site are deleted. When the rearrangement occurs at a site within that described by a particular probe, rearrangement is demonstrated as an altered size of the hybridizing fragment. In the event of a rearrangement at a site between those described by two probes, then the upstream probe detects deletion and the downstream probe shows germline hybridization. The rearrangement patterns of the TCR- α gene are summarized in Tables II and III and representative Southern blots are shown in Fig. 2. Among the four patients classified as stage I, only patient 4 showed rearrangement of the TCR- α gene. These rearrangements were detected with the J α F and J α G probes. The remaining three patients (patients 1-3) and the three patients (5-7) in stage II had no rearrangements.

Three samples from patients 9, 11, and 12 classified as stage III demonstrated TCR- α gene rearrangements. In two of these (patients 9 and 12), a single rearranged band was observed after hybridization with the J α G probe. In patient 11, the J α F and J α G sequences were deleted with retention of the

J α E sequences on both alleles indicating the usage of J α segments located between the J α E and J α F sequences. After Bam HI digestion, patient 8 showed a rearranged band correspond-

Table III. Patterns of T Cell Receptor α Gene Rearrangements*

Patient No.	Stage	J α C	J α D	J α E	J α F	J α G
4	I	G	G	G	R/G	D/R
9	III	G	G	G	G	R/G
11	III	G	G	G	D/D	D/D
12	III	G	G	G	G	R/G
15	IV	G	D/G	D/G	D/G	D/D
16	IV	G	G	G	R/G	D/G
17	IV	G	G	R/G	D/R	D/D
18	IV	G	G	G	G	R/D
19	IV	G	G	G	G	D/D
HSB2		G	G	G	G	R/D
CEM		G	G	R/D	D/D	D/D
MOLT4		G	D/D	D/D	D/D	D/D

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

and/or rearranged bands corresponding to the $J\alpha G$ probe with retention of the $J\alpha F$ sequences on both alleles. In patient 15, the $J\alpha D$ sequences and $J\alpha$ regions located upstream of these were deleted on one allele and the $J\alpha G$ sequences were deleted on the other allele. Patient 16 showed a rearranged band that hybridized with the $J\alpha F$ probe, whereas the $J\alpha G$ sequences were deleted on a single allele. In patient 17, rearranged bands corresponding to the $J\alpha E$ and $J\alpha F$ probes were observed.

All three leukemic T cell lines examined also had TCR- α gene rearrangements on both alleles. The CD3⁻ T cell line HSB2 only expressed the CD2 and CD7 antigens suggesting derivation from an immature stage. These cells showed a single rearranged band corresponding to the $J\alpha G$ probe with a deletion of the other allele. CEM and MOLT4 cells also did not express the CD3 antigen and demonstrated rearrangements of both alleles of the TCR- α gene. In CEM, a rearranged band and a deletion corresponding to the $J\alpha E$ probe were observed. MOLT4 showed complete deletion of regions corresponding to the $J\alpha D$ probes with biallelic retention of the $J\alpha C$ sequences.

Overall, among the CD3⁻ cells, one (14%) of seven stage I and II patients and all three leukemic T cell lines showed rearrangements of the TCR- α gene on both alleles. Among the stage III patients, three (43%) had rearrangements. In two of these, a single allele was involved and in the remaining patient both alleles were rearranged. All five stage IV patients had TCR- α gene rearrangements and four of five showed rearrangements on both alleles.

RNA analysis. To analyze the transcription of TCR genes, Northern blot analyses were performed on total cytoplasmic RNA from 11 patients (three stage I, four stage III, and four stage IV patients) where sufficient material was available and from all three T cell lines. The results are summarized in Table II and representative blots are shown in Fig. 3.

Transcription of the TCR- γ gene was analyzed in 10 patients and the three T cell lines. Two RNA samples (patients 11 and 19) contained no TCR- γ transcripts, while 1.7-kb TCR- γ transcripts were observed in all other samples. In patient 1, a 1.3-kb TCR- γ transcript was detected in addition to the 1.7-kb transcript. All three T cell lines also expressed 1.7-kb TCR- γ transcripts. However, the levels were very low in CEM and MOLT4.

All 11 patients and three T cell line samples contained 1.0- and/or 1.3-kb transcripts of the TCR- β gene. The 1.3-kb transcript is derived from VDJ β recombination, while the 1.0 kb does not contain V region transcripts (39). In two (patients 2 and 4) stage I patients, 1.3-kb transcripts were present and in the remaining stage I patient (patient 1), only a 1.0-kb transcript was observed. 1.3-kb TCR- β transcripts were present in seven of eight patients classified as stage III and IV, while one (patient 10) possessed only a 1.0-kb transcript.

Hybridization with the $C\alpha$ probe revealed 1.3- and/or 1.6-kb transcripts in 8 of the 11 patients. The 1.6-kb transcript encodes the complete TCR- α polypeptide, however, the 1.3-kb transcript is presumably immature (40). No TCR- α transcripts were observed in the three CD3⁻ patients (stage I) tested nor in the CD3⁻ cell lines. In contrast, RNA from all eight CD3⁺ patients contained 1.6-kb transcripts, however, the levels of TCR- α transcripts varied compared to TCR- β transcripts and were relatively low in patients 10, 12, and 17.

Finally, one component of the CD3 complex, CD3- γ was

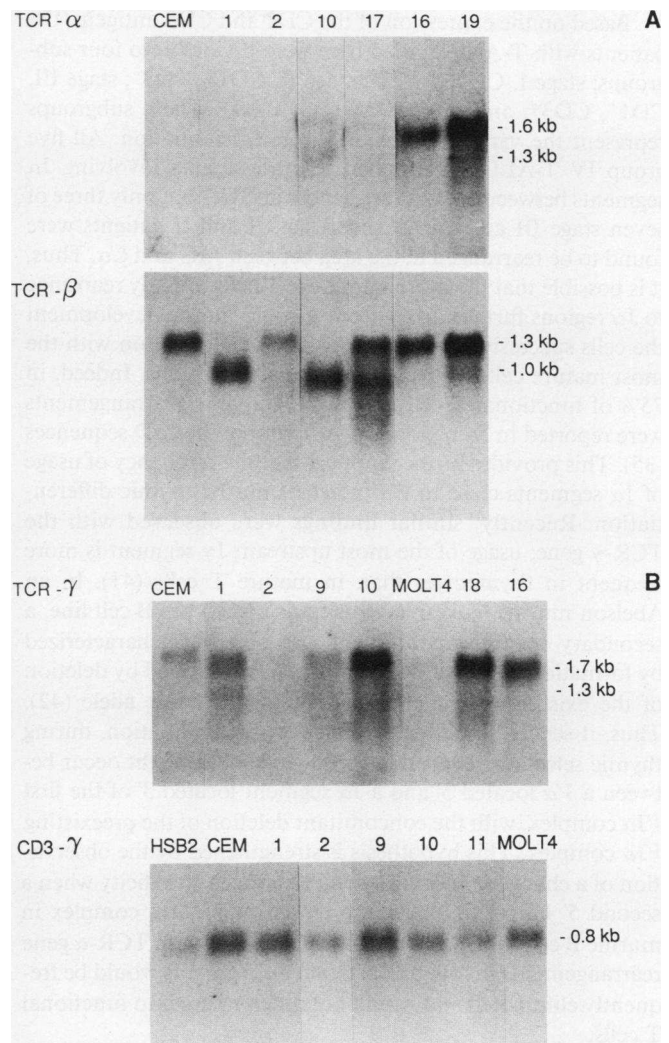


Figure 3. Expression of TCR genes. The TCR- α and β genes (A) and the TCR- γ and CD3- γ genes (B) were examined in patients with T cell acute lymphoblastic leukemia and in leukemic T cell lines. The patient numbers are noted above each lane. Sizes of transcripts are indicated on the right side of the gels. Blots were hybridized sequentially after removal of hybridized probes. Complete removal was confirmed by another exposure before the next hybridization.

analyzed. 0.8 kb CD3- γ transcripts were present in all patients and all T cell lines tested, regardless of whether or not the cells expressed CD3 on the cell surface.

Discussion

In an attempt to delineate the hierarchy of TCR gene activation, we have analyzed TCR- α gene configuration in 19 T-ALL and 3 leukemic T cell lines. Using newly developed genomic $J\alpha$ probes that cover about 80 kb of the $J\alpha$ region upstream from $C\alpha$, TCR- α gene rearrangements were observed in both CD3⁺ and CD3⁻ cells. Use of these probes demonstrated that TCR- α gene rearrangement occurs at an early stage of differentiation but is not always accompanied by expression of the TCR- α gene.

Based on the expression of the CD1 and CD3 antigens, the patients with T-ALL studied here were divided into four subgroups; stage I, CD1⁻, CD3⁻; stage II, CD1⁺, CD3⁻; stage III, CD1⁺, CD3⁺; and stage IV, CD1⁻, CD3⁺. These subgroups represent the various stages of T cell differentiation. All five group IV T-ALL patients had rearrangements involving J α segments between the J α G sequences and C α , but only three of seven stage III and one of seven stage I and II patients were found to be rearranged in the area between J α G and C α . Thus, it is possible that the more immature T cells initially rearrange to J α regions furthest away from C α and during development the cells successively rearrange in a 5' to 3' direction with the most mature cells utilizing the 3' most J α regions. Indeed, in 75% of functional T cell clones TCR- α gene rearrangements were reported in J α regions downstream of the J α D sequences (35). This provides further support that the frequency of usage of J α segments close to C α increases during thymic differentiation. Recently, similar findings were observed with the TCR- γ gene; usage of the most upstream J γ segment is more frequent in thymocytes than in mature T cells (41). In an Abelson murine leukemia virus transformed pre-B cell line, a secondary rearrangement event was observed, characterized by formation of a new DJ_H complex accompanied by deletion of the existing DJ_H rearrangement on the same allele (42). Thus, it seems that after the first VJ α recombination, during thymic selection, a secondary rearrangement might occur between a V α located 5' and a J α segment located 3' of the first VJ α complex, with the concomitant deletion of the preexisting VJ α complex. This hypothesis is strengthened by the observation of a change in immunoglobulin antigen specificity when a second 5' V_H recombined into an existing VDJ_H complex in murine B cells (43, 44). Alternatively, T cells with TCR- α gene rearrangements involving upstream J α segments would be frequently eliminated and would not differentiate into functional T cells.

Four stage III patients showed a germline configuration of the TCR- α gene despite the expression of the CD3 antigen on the cell surface. Expression of the CD3 complex on the cell surface may require the presence of the α/β or γ/δ heterodimer (26–30). Recently, novel constant genes, candidates for C δ , were discovered 3' to V α and 5' to C α in mice (45) and in humans (46). In humans, this constant gene was located ~ 90 kb 5' to C α . Since the J α probes used in this study were not capable of detecting TCR- α gene rearrangements occurring between putative C δ and J α G sequences, it is possible that these cells had TCR- α gene rearrangements in this region. Alternatively, the γ/δ heterodimer may be expressed together with the CD3 antigen on the cell surface and the rearrangements of the TCR- δ gene may have occurred with retention of germline configuration of the TCR- α gene. It will be important to examine these patients using additional probes as they become available in order to delineate the mechanism of TCR- α and TCR- δ gene rearrangements.

In T-ALL with the most immature phenotype (stage I), patient 4 had rearrangements of the TCR- α gene as did the cell lines HSB2, CEM, and MOLT4, which are representative of immature stages of T cell development. In these samples, no TCR- α transcripts were expressed in spite of TCR- α gene rearrangement suggesting that these rearrangements alone were not sufficient for the expression of the TCR- α gene. In con-

trast, all T-ALL tested, as well as the three T cell lines, had both rearrangements and transcripts of the TCR- β gene and no discordance was observed. Thus, the system for TCR- α gene activation appeared quite different from that for activation of the TCR- β gene. One explanation for the discordance between rearrangement and expression of the TCR- α gene is that the type of rearrangement observed may be incomplete. Alternatively, activation of a regulatory system may be required for TCR- α expression. Shackelford et al. observed that TCR- α transcripts were induced by TPA in CD3⁻ subclones of CEM (47). Moreover, induction of TCR- α transcripts by TPA are enhanced by incubation with cycloheximide (unpublished data), indicating that specific proteins may regulate the transcription of the TCR- α gene and/or the degradation of TCR- α transcripts.

In murine fetal tissue, TCR- γ transcription precedes transcription of TCR- β and TCR- α , thereafter, it decreases in inverse proportion to TCR- α transcription during thymic development (21–26). In this study, however, most of stage III and IV T-ALL had TCR- γ transcripts. These T cells may still be immature considering the presence of TCR- γ transcripts. Alternatively, leukemic transformation may have influenced transcription and preservation of the TCR- γ gene. Patient 1, classified as stage I, contained a 1.3-kb TCR- γ transcript in addition to the 1.7 kb TCR- γ transcript. A transcript from an unrearranged V γ gene has also been reported in the leukemic T cell line PEER (48). It is possible that the 1.3-kb TCR- γ transcript may be the product of a germline gene and reflect that the TCR- γ gene remains accessible for further rearrangements.

All T-ALL tested and the three T cell lines had TCR- β transcripts of 1.0 and/or 1.3 kb. The 1.3-kb transcript is derived from VDJB recombination, while the 1.0 kb does not contain V region transcripts (39). One CD3⁺ T-ALL (patient 10) expressed only the 1.0 kb TCR- β transcript and TCR- γ transcripts were detected. In this patient, the TCR complex associated with the CD3 molecule may be comprised of an alternative receptor protein, i.e., γ/δ heterodimer. A low level of TCR- α transcripts was also observed. However, we cannot exclude the possibility of the contribution of contaminating nonleukemic mature T cells.

To further delineate the processes involved in the formation of the TCR complex, we have also analyzed gene expression of CD3- γ . The most immature T-ALL (stage I) as well as all other T-ALL tested had CD3- γ transcripts. The transcription of the CD3- γ gene may represent one of the earliest events, similar to CD3- δ and CD3- ϵ , in T cell differentiation (27–29). Thus, the detection of CD3- γ provides a useful marker for assignment of cell lineage in leukemias of ambiguous phenotype.

In summary, most T-ALL and leukemic T cell lines tested expressed TCR- γ transcripts and all samples expressed TCR- β and CD3- γ transcripts. TCR- α transcripts were confined to CD3⁺ T-ALL and the majority of CD3⁺ T-ALL also showed rearrangement of the TCR- α gene. However, TCR- α gene rearrangements were observed even in CD3⁻ T-ALL and in T cell lines containing no TCR- α transcripts. These leukemic cells may represent a transient stage between rearrangement and expression and provide an opportunity for determining the mechanism regulating the expression of the TCR- α gene.

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