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Research Article

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T Cell Receptor α-, β-, and γ-Genes in T Cell and Pre-B Cell Acute Lymphoblastic Leukemia

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Abstract

We examined α-, β-, and γ-T cell receptor (TCR) gene activation within acute lymphoblastic leukemias (ALLs) that represent early stages of B and T cell development. We wished to determine if TCR rearrangement and expression was lineage restricted, showed any developmental hierarchy, or could identify new subsets of T cells. Rearrangement of γ and β TCR genes occurred early in development but in no set order, and most T-ALLs (22/26) were of sufficient maturity to have rearranged both genes. T-ALLs preferentially rearranged $C_\gamma 2$ versus the $C_\gamma 1$ complex; no preference within the β locus was apparent. Once rearranged, the β TCR continued to be expressed (11/13), whereas the γ TCR was rarely expressed (3/14). The α TCR was expressed only in more mature T-ALLs (8/14) that usually displayed T_3. The 3A-1 T cell associated antigen appeared earliest in development followed by T11 and T3. Within pre-B cell ALL a higher incidence of lineage spillover was noted for γ TCR rearrangements (8/17) than for β rearrangements (3/17). This also contrasts with the only occasional rearrangement of immunoglobulin (Ig) heavy chains (3/25) in T-ALL. However, in pre-B ALL the pattern of γ TCR usage was distinct from that of T cells, with the $C_\gamma 1$ complex utilized more frequently. Almost all ALLs could be classified as pre-B or T cell in type by combining Ig and TCR genes with monoclonal antibodies recognizing surface antigens, although examples of lineage duality were noted. Unique subpopulations of cells were discovered including two genetically uncommitted ALLs that failed to rearrange either Ig or TCR loci. Moreover, two T lymphoblasts were identified that possessed the T_3 molecule but failed to express α plus β TCR genes. These T-ALLs may represent a fortuitous transformation of T cell subsets with alternative T_3-Ti complexes.

Introduction

The antigen-specific T cell receptor genes provide the first universally applicable markers of T cell clonality. The characterization of the T cell receptor (TCR)[1] loci in lymphoid neoplasms should improve our understanding of lineage commitment, clonality, stage of differentiation, and T cell associated chromosomal translocations. In this study we examined rearrangement and expression of TCR genes within acute lymphoblastic leukemias of both T cell and pre-B cell type. We wished to determine whether the activation of α, β, and γ TCR genes was lineage restricted and whether this was useful in placing early lymphoid progenitors into T cell versus B cell lineages. Moreover, we sought to determine if there was a hierarchy of α, β, and γ TCR gene utilization, if it correlated with T cell surface antigen expression, and whether this information could pinpoint the state of differentiation of individual T cell ALLs.

We previously demonstrated that the non-T ALLs that lacked T cell surface antigens were a developmental series of B cell precursors (1–3). All of these pre-B ALLs had rearranged immunoglobulin (Ig) heavy (H) chain genes and ~ 40% had progressed to the later developmental step of rearranging their κ or λ light (L) chain genes. Coordinate with this hierarchy of Ig gene rearrangements was an ordered expression of B cell associated surface antigens in which B4 and HLA-DR were present on the most immature cells, the common acute lymphoblastic leukemia antigen (CALLA) followed, and B1 was present on the most mature cells. Approximately 20% of ALLs bore T cell associated surface antigens (4, 5), uniformly retained germline Ig L-chain and usually (90%) germline Ig H chain genes (2), and were consequently felt to represent T cell lineage ALLs. A number of T cell associated surface antigens have been identified, however none of these are malignancy restricted nor do they serve as clonal markers. Consequently, we investigated the TCR genes in T-ALL to determine if an analogous cascade of rearrangements occurs in pre-T as it does in pre-B development.

The classic antigen specific T cell receptor is a Ti heterodimer of the α plus β TCR chains that is complexed intramembranously to form the T3/Ti molecular complex (6). T3-Ti expression is noted only in the most mature thymocytes and peripheral T cells (7, 8). The human β TCR gene is located on chromosome 7 at band q35 (9) and is comprised of two constant (C_β) segments, 13 joining (J_β) segments, two or more diversity (D_β) segments, and ~ 100 variable (V_β) gene segments (10–12). Rearrangements of this locus are readily detected and intermediate (D/J) 1.0 kilobase (kb) and complete (V/D/J) 1.3 kb transcripts can be discerned (13–18). The α TCR locus is found at 14q11 and is

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1. *Abbreviations used in this paper:* CALLA, common acutae lymphoblastic leukemia antigen; EBV, Epstein Barr virus; sRBC, sheep red blood cell; TCR, T cell receptor, tdt, terminal deoxynucleotidyl transferase; WBC, white blood cells.

particularly prone to interchromosomal recombination in T cell ALLs (19–22). It consists of one C_α region, an exceptionally long stretch of J_α segments that may contain 50–100 J regions spread over more than 70 kb. No diversity segments are known to exist in the α TCR locus, but there are probably 40 or more V_α regions (23–25). While the markedly long stretch of J_αs makes it currently rather impractical to search for rearrangements in all cells, RNA transcripts of complete recombinations (1.6 kb) and truncated forms (1.3 kb) can be assessed (23). In addition to the α- and β-chain that contribute to the Ti heterodimer, at least one other TCR gene, γ is known to exist (26–29). This rearranging determinant is found on chromosome 7 at band p15 (30). In humans two C_γs are present, together with three or four J_γs, and perhaps in the range of 10 V_γ segments. Despite having a similar organization to the other TCR genes the precise function of this locus is uncertain (31, 32). Because of this, we wished to assess its rearrangement and expression in T-ALLs to gain potential insights into its role in T cell development.

Developmental studies in the mouse suggested a hierarchy of TCR gene activation in which γ TCR expression might precede the β TCR. γ TCR RNA decreased dramatically during intrathymic maturation, while β RNA remained constant, and α RNA increased (6, 7, 33–36). We sought to determine whether the T-ALLs represented a population of cells early enough in pre-T development to reveal any ordered usage of TCR genes in man. Moreover by examining T3 and other cell surface antigens together with α, β, and γ we also sought potentially new subpopulations of T cells. The comparison of Ig and TCR genes in pre-B and T cell ALLs was performed to assess the lineage restriction of these developmental events and to determine if any dual-lineage or clearly uncommitted lymphoid progenitor cells existed.

Methods

ALLs examined. Leukemic cells were obtained by Ficoll-Hypaque gradient centrifugation of bone marrow aspirates or leukapheresis obtained lymphocyte fractions from peripheral blood of patients with ALL at either diagnosis or relapse. Samples were available from 23 patients categorized as T cell ALL and 17 patients classified as pre-B cell ALL. In addition, three established T-ALL cell lines (HSB, CEM, and RPMI 8402) and four pre-B cell lines (RS, Nalm-6, Nalm-1, and Reh) were also examined (2, 37, 38). Also studied were examples of more mature B cell neoplasms including a B cell chronic lymphocytic leukemia, five Burkitt lymphoma cell lines (IARC LY47, IARC LY67, IARC JI, IARC LY66, Raji), and six B cell lymphoma cell lines (SU-DHL-4, SU-DHL-6, SU-DHL-7, SU-DHL-8, Balm-1, Balm-4) (39–41). Moreover, 14 clonal Epstein Barr Virus transformed B cell lines were utilized. The Hut 102 cell line was utilized as an example of a mature T cell (42), while K562 represented an erythroleukemia (43), HL-60 a promyelocyte (44), and SU-DHL-1 a histiocyte (40).

ALLs were initially classified as T cell type if they possessed T cell associated antigens (3A-1, and at times T11 and T3), lacked B cell associated antigens (B4 and B1), and retained germline Ig L chain genes. In contrast, ALLs were initially classified as pre-B cell in type if they lacked T cell associated antigens, demonstrated B cell associated antigens (B_4, HLA-DR, and at times B_1), possessed rearranged Ig H chain genes and at times rearranged L chain genes (2, 3). Complete examination of all such markers were performed whenever possible. Limited numbers of leukemic cells prevented examination of all markers in some cases and such cells were classified as pre-B or T based on available data (Tables I, II).

TCR gene configuration. High molecular weight DNA was extracted from leukemic cells or cell lines as well as total white blood cells (WBCs) from normal individuals. 15 μg of genomic DNA was digested to completion with 5–10 U/μg DNA of the appropriate restriction endonuclease, sized fractionated on 0.6–1.0% agarose gels, and transferred to nitrocellulose paper (2, 45). Such genomic blots were hybridized to ^{32}P-labeled DNA probes prepared by a random priming method (46). These Southern blots were washed at the appropriate stringency and autoradiography was performed.

The C_β probe was a 700 base pair (bp) Eco RI cDNA fragment containing the murine C_β region and is capable of hybridizing to both human C_β regions under routine stringency (14). In the germline form both J_β-C_β1 and J_β-C_β2 are located on a single 23 kb Bam HI fragment as indicated in Fig. 1 *A*. Thus, rearrangements of either the C_β1 or C_β2 complex should alter the size of the Bam HI fragment. To discern the identity of such rearrangements Eco RI and Hind III digests were used. In the germline form C_β1 occupies an 11-kb Eco RI fragment and C_β2 a 4-kb fragment. The 11-kb fragment contains the J_β1 region and rearranges when the C_β1 complex is used, whereas the 4-kb fragment lacks J_β2 and never rearranges. Occasionally an 8-kb Eco RI band is noted even in germline sources of tissue. This represents either an Eco RI resistant site or perhaps at times a restriction fragment length polymorphism, and cannot be assumed to be a somatic rearrangement. In contrast, Hind III digestion is capable of detecting rearrangements of the C_β2 locus. J_β-C_β2 occupy an 8.0-kb Hind III fragment that rearranges. C_β1 is found on a 3.5-kb Hind III fragment that is deleted when a V_β region rearranges into the C_β2 locus.

The C_γ probe was a 300 bp PstI-Bam HI fragment of a human C_γ containing subclone (30). It was routinely hybridized to Bam HI digested human genomic DNA and those cells displaying germline patterns were also examined with Sac I and PvU II digested DNA. In its germline configuration the C_γ locus displays a 15 kb Bam HI fragment containing C_γ1 and a 12.5-kb fragment with C_γ2 (28). Bam HI detects rearrangements of either the C_γ1 or C_γ2 locus. A single set of V_γ genes appears to exist 5′ of C_γ1 as diagrammed in Fig. 3 *B*. Hence, rearrangements into the C_γ2 locus result in the deletion of the 15-kb fragment.

TCR gene expression. In addition RNA was extracted from 14 T cell ALL leukemic samples and cell lines, 4 pre-B cell lines, 4 mature B cell lines, Hut 102, HL-60, and K562. Total cellular RNA was prepared by a guanidine thiocyanate method and 10 μg was denatured in formamide, electrophoresed on agarose-formaldehyde gels, and transferred to nitrocellulose paper (47). Northern blots were hybridized with the C_γ and C_β probes described as well as with a 1.1 kb Eco RI fragment containing Cα from a human α TCR cDNA clone (23). In addition all Northern blots were hybridized with a γ-actin probe to insure that equal amounts of intact, hybridizable RNA was present in each lane (48). Blots were stripped clean of probe by washing in 0.1% sodium dodecyl sulfate (SDS) at 70°C for 20 min and autoradiography was performed to insure a complete wash off.

Ig gene configuration. Human Ig gene probes were used to assess the status of H chain as well as κ and λ L chain genes. A human genomic 6.0 kb Bam HI-Hind III fragment containing the entire J_H region was used to assess rearrangements in both Bam HI and Hind III digested DNA (49). κ gene rearrangements were detected in Bam HI digested genomic DNA with a 2.5-kb germline Eco RI C_κ containing fragment (50). A 0.8 kb BglII-Eco RI fragment containing C_λ was capable of detecting any λ rearrangements in Eco RI digested DNA (50).

Phenotypic characterization. When adequate numbers of leukemic cells existed, they were assessed for cell surface antigens. One million viable cells per test were reacted with a predetermined concentration of a mouse monoclonal antibody at 4°C for 30 min, washed twice, and then reacted with a fluorescein-labeled goat anti-mouse Ig second antibody. After three washes the cells were assessed by flow microfluorometry with a fluorescence-activated cell sorter (51). Cells were reacted with normal mouse serum and the fluorescein-labeled second antibody as a background control. A fluorescence profile of each antibody was produced and the appropriate background subtracted. This generated an estimate

of the percentage of cells within the leukemic population that bore the antigen. Alternatively, a few samples were assessed by direct visualization with conventional immunofluorescence microscopy. If > 50% of the cells bore the antigen, the monoclonal antibody was scored as positive (+), whereas < 10% was scored as negative, and values between 10 and 50% were listed in Tables I and II.

Monoclonal antibodies used here include the 3A-1 monoclonal that recognizes CD-7 (52), T11 (CD-2) (53), T3 (CD-3) (54), T4 (CD-4) (54), T8 (CD-8) (54), T6 (CD-1) (54), B4 (CD-19) (3), B1 (CD-20) (3), J5 (CALLA, CD-10) (55), and I2 (HLA-DR) (56). In addition many of the cells were analyzed with BA-1 (CD-24) (57), BA-2 (CD-9) (58), T12 (CD-6) (54), and for terminal deoxynucleotidyl transferase (tdt) (data not shown). Cells lacking Ig and TCR gene rearrangements were examined for myeloid-associated antigens with MO2 (59), LeuM1 (60), and LeuM3 (61).

Results

β TCR gene configuration in T cell ALL. The vast majority of patients cells that would phenotypically be classified as T cell type ALL possessed rearranged β TCR genes (23/26) (Table I, Fig. 1). Most cells rearranged both alleles (19/23) of their β-chain genes, while one exceptional case displayed an unanticipated three rearrangements of β TCR restriction fragments (Fig. 1 *B*). Cytogenetic examination of this cell revealed numerous abnormalities including a translocation, t(7:11) (q35-36; q14) at or near the band containing the β TCR, 7q35. Such an extra-rearrangement could conceivably be created by a chromosomal translocation breakpoint that fell within the Cβ TCR region. Alternatively, this might represent clonal evolution or a duplicated TCR gene that subsequently rearranged. This leukemia provides an opportunity to explore these possibilities. By utilizing three restriction endonucleases, Bam HI, Eco RI, and Hind III, it is possible to discriminate between rearrangements of $C_\beta 1$ versus the $C_\beta 2$ regions (Fig. 1 *A–D*). All three rearrangements in patient 4 were of the $C_\beta 2$ complex (Fig. 1 *B*). In contrast both rearranged alleles of CEM were $C_\beta 1$ and the $C_\beta 2$ locus was germline (Fig. 1 *C*). Rearrangement of $C_\beta 1$ and $C_\beta 2$ loci were not exclusive, however, as patient 2 and several others revealed a rearrangement of $C_\beta 1$ on one allele and $C_\beta 2$ on the other (Fig. 1 *D*). Overall, there was no preferential usage of $C_\beta 1$ versus $C_\beta 2$. Moreover, there was no correlation between $C_\beta 1$ versus $C_\beta 2$ usage and the cell surface antigens expressed or any postulated developmental status (Table I).

β TCR gene expression in T cell ALL. Most T cell ALLs with rearranged β TCR genes also expressed this locus (11/13) (Table I and Fig. 1 *E*). Nearly all cases that expressed β produced the full length 1.3-kb transcript (10/11) signifying the presence of a completed V/D/J rearrangement. Four cells expressed the 1.0-kb RNA compatible with the product of an intermediate D/J rearrangement. Three of these also expressed a full sized transcript while one T-ALL possessed only the truncated 1.0-kb form. β TCR gene expression was not completely lineage restricted as Raji, a Burkitt lymphoma cell line, produced a truncated 1.0 kb β transcript (not shown). Four of the T cells that expressed β failed to express the α TCR supporting a sequential use of these genes.

α TCR gene expression in T cell ALL. The extraordinary long stretch of genomic J_α regions makes it currently rather impractical to conclusively determine the status of α TCR rearrangements in every T cell. For this reason we concentrated upon α TCR locus expression. In contrast to the β TCR expression, slightly over one-half (8/14) of the T-ALLs expressed the α TCR locus (Table I, Fig. 2). Most of these T cells (7/8) possessed the full length 1.6 kb transcript. Most T-ALLs with α TCR expression were of the most mature type displaying surface T3. Most of these (7/8) also expressed a 1.3-kb β mRNA and presumably possessed a functional T3-Ti complex. One important exception expressed γ and a unique 2.0 kb α TCR gene but not β (patient 7). In addition one case expressed α, β, and γ TCR genes (patient 4). No individuals expressed α alone, compatible with a developmental sequence where α-chain production is a later event in T cell ontogeny. α TCR gene expression is not lineage restricted as RPMI 8432 a B cell lymphoblastoid line (Fig. 2), SU-DHL-6 a mature B cell and RS a pre-B cell (not shown) were all noted to make a truncated 1.3-kb form of the α transcript.

γ TCR gene rearrangement in T cell ALL. Most T cell ALLs (23/26) have rearranged their γ TCR locus (Table I, Fig. 3 *A, B*). Moreover the vast majority of T-ALLs that utilized γ, rearranged both alleles (17/23). Examples (4/23) occurred in which either one allele had been deleted or two separate rearrangements fortuitously created the same sized Bam HI fragment. Inspection of γ TCR rearrangement in T-ALLs revealed that apparently all had rearranged into the $C_\gamma 2$ complex. In general T-ALLs show no remaining $C_\gamma 1$ containing 15.0 kb Bam HI germline fragment and no remaining $C_\gamma 2$ containing 12.5 kb Bam HI germline fragment (Fig. 3 *A*). This finding results from rearrangements into the $C_\gamma 2$ locus on both alleles (Fig. 3 *B*). This rearrangement pattern in T cells is different than the γ TCR rearrangements seen in pre-B cells (Fig. 3 *D*) as will be presented below. Unusual patterns included patient 16 with three rearranged C_γ fragments. Unfortunately, no cytogenetic data is available on patient 16. Overall 22 of 26 T-ALLs rearranged both their γ as well as β TCR loci and the important exceptions will be detailed later.

γ TCR gene expression in T cell ALL. Despite the frequent rearrangement of γ TCR genes in T-ALLs this gene was only occasionally transcribed at detectable levels by Northern blot analysis of total RNA. Only 3 of 14 T ALLs examined (patients 4 and 7 and the HSB cell line) revealed any γ transcripts (Fig. 3 *C*, Table I). All three of these T cells possessed γ TCR rearrangements.

γ Without β, β without γ TCR rearrangement. Several cells possessed rearrangement patterns that argue against any strictly fixed order to β and γ TCR gene rearrangement. Two rearranged γ TCR genes in the presence of germline β TCR genes were evident in patient 7 (Fig. 4 *A*, Table I). Curiously, this cell demonstrates a rare γ TCR pattern for a T cell in that a germline 12.5 kb $C_\gamma 2$ band is retained arguing that one of the rearrangements involves the $C_\gamma 1$ locus. It also expresses γ TCR RNA (Fig. 3 *C*). This cell also displayed a germline pattern for Ig heavy chain and κ and λ light chain loci. However, this T-ALL did not appear to represent an early cell in differentiation as it expressed a 2.0-kb α TCR and displayed surface T_3 (Fig. 2, Table I). This illustrates that β rearrangement is not required for α TCR gene activation. Moreover, the presence of cell surface T_3 in the absence of β TCR gene rearrangement or expression indicates the presence of a T3 receptor complex without a classic α, β heterodimer.

The opposite TCR gene configuration was demonstrated by patient 3 which displayed a β-chain rearrangement but germline γ TCR genes (Fig. 4 *B*, Table I). The germline 15.0 and 12.5

Table I. T Cell ALL

	TCR Genes						Ig Genes				RNA Expression			Phenotype*								
Patient No.	$C_T\gamma$	No.	$C_T\beta$	$C_T\beta$	$C_T\beta$	No.	J_H	No.	C_k	C_λ	$C_T\alpha$	$C_T\beta$	$C_T\gamma$	3A1	T11/SRBC-R	T6	T4	T8	T3	B4	DR	Calla	B1
			Bam HI	Eco RI	Hind III																		
1	R	2	R	D $C_\beta1$	R $C_\beta2$	2	G		G	G	N	E 1.0	N	+	−	−	+	+	−	−	−	−	−
2	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G				+	+				31	−			−
3	G		R		R $C_\beta2$	1	G		G					+	35	−	−	35	−		49	−	
4	R/D	1/1	R	D $C_\beta1$	R $C_\beta2$	3	G		G	G	E 1.6	E 1.3	E 1.5		+	+	+	+	25	−	20	37	27
5	G		G	G	G		G		G	G				+	−	−	−	−	−	−	−	−	−
6	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G				+	+	+	+	+	23	−	−	−	−
7	R	2	G	G	G		G		G	G	E 2.0	N	E 1.5	+	+	32	22	+	+	−	−	−	−
8	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G				+	+		+	+	−	−	−	−	−
9	R	2	R	R $C_\beta1$	G	2	G		G	G	E 1.6	E 1.0/1.3	N	+	+	+	−	−	+	−	46	−	−
10	R/D	1/1	R	R $C_\beta1$	R $C_\beta2$	2	G			G				+	+	−			+	−	−	−	−
11	R	2	R	R $C_\beta1$	R $C_\beta2$	2	R	1	G	G	E 1.6	E 1.0/1.3	N	+	+	−	−	−	−	12	−	−	13
12	R	1	R			2								+	+	+	13	+	−	−	−	14	13
13	R/D	1/1	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G	E 1.6	E 1.0/1.3	N	22	+	28	39	+	+	−	−	−	−
14	R	2	R			1	G				E 1.6	E 1.3	N	+	+	+	+	+	+	−	−	−	+
15	R	2	R	R $C_\beta1$		2	G		G	G	E 1.6	E 1.3	N	+	+	+	+	+	+	−	−	−	15
16	R	3	R	G	R $C_\beta2$	1	R	2	G	G	N	N	N	+	+	−			−	−	44	−	−
17	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G	N	E 1.3	N	+	+	30	+	+	+	−	−	−	12
18	G		G	G	G		G		G	G				+	+				−			−	
19	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G				+	+	24	20	+	+	−	40	−	−
20	R/D	1/1	R	D $C_\beta1$	R $C_\beta2$	2	G		G	G				+	+							−	
21	R	2	R	D $C_\beta1$	R $C_\beta2$	2	G		G	G				+	+				−			−	
22	R	2	R			2	G			G	N	N	N	+	−	−	−	41	−	−	14	−	−
23	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G			G				+	+	+	+	+	+	−	−	−	−
Cell line																							
8402 T	R	2	R	R $C_\beta1$	R $C_\beta2$	2	G		G	G	N	E 1.3	N	+	+	−	−	−	−		−	−	
CEM	R	2	R	R $C_\beta1$	G	2	G		G	G	E 1.6	E 1.3	N	+	+	+	+	+	−		−	42	
HSB	R	2	R	R $C_\beta1$	G	2	R	1	G	G	N	E 1.3	E 1.5	+	+	−	−	−	−		−	−	

* R, rearranged, D, deleted, G, germline, No., number of alleles, E, expressed. Data in which a (−) is < 10% of cells showing reactivity, a (+) is > 50% reactivity, and specific values are given for the 10–50% range.

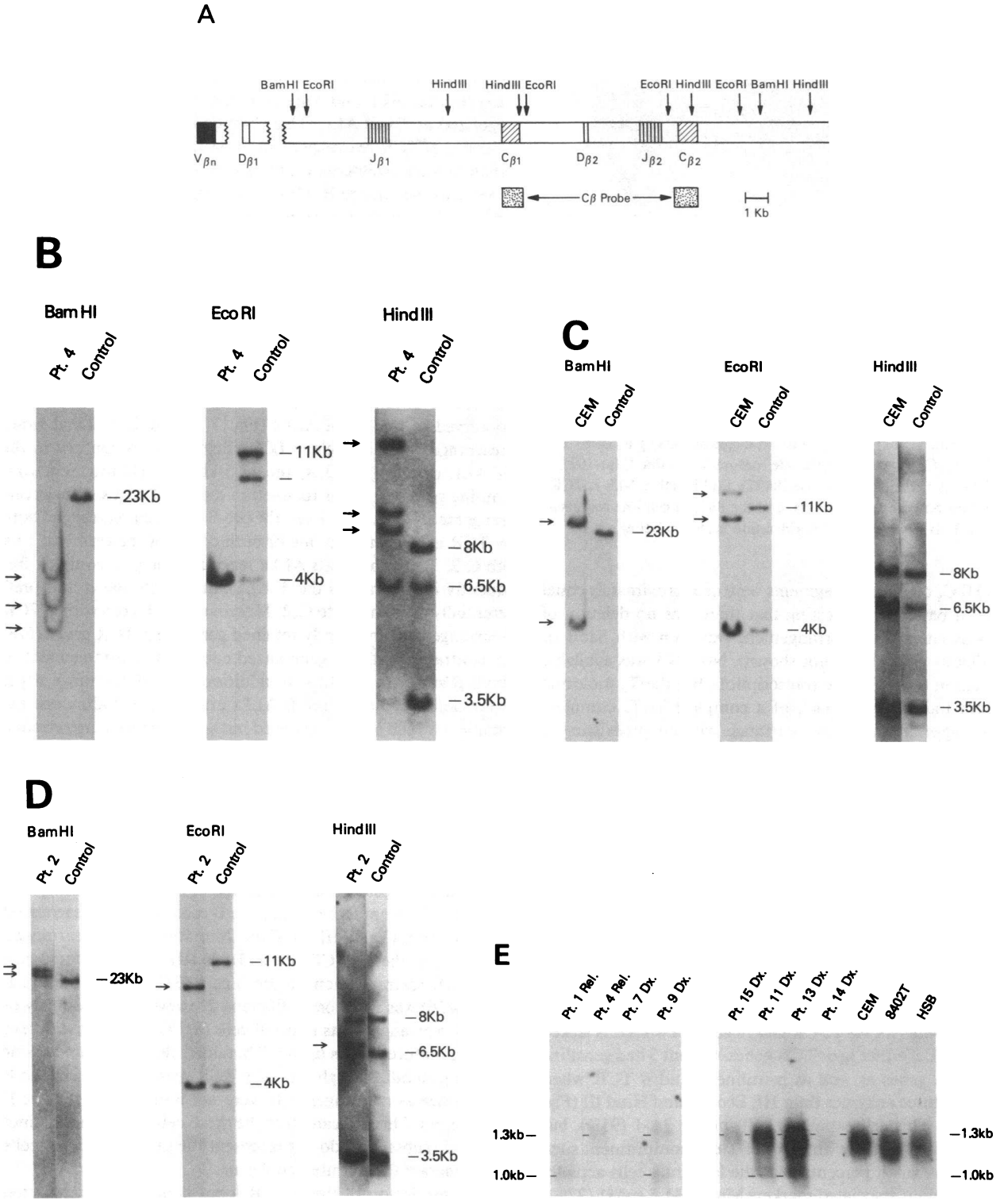

Figure 1. (*A*) Schematic presentation of the human β TCR locus and the utilized Bam HI, Eco RI, and Hind III restriction sites. Variable (V), diversity (D), joining (J), and constant (C) regions are shown. (*B*) β TCR configuration in patient 4 that shows three rearrangements (*arrows*) of the $C_\beta 2$ complex when compared to the germline configuration (*dash marks*) revealed by the control. The additional 8 kb Eco RI fragment is seen in the control and may represent an Eco RI resistant site. (*C*) β TCR pattern of CEM demonstrates two rearrangements of the $C_\beta 1$ complex. (*D*) β TCR pattern of patient 2 illustrates one rearranged $C_\beta 1$ and one rearranged $C_\beta 2$ allele. (*E*) Northern blot analysis of total RNA from T ALL patients at diagnosis (Dx) or relapse (Rel) and three T-ALL lines. Full length (VDJ) 1.3 kb and intermediate (DJ) 1.0 kb transcripts are noted.

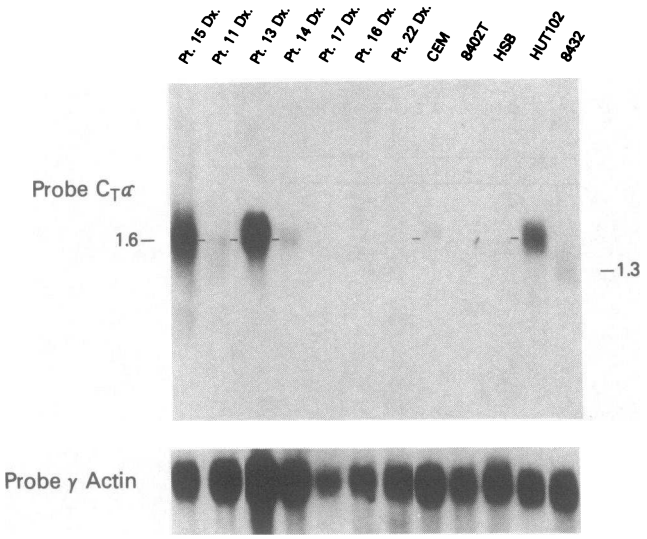

Figure 2. Northern analysis of T-ALL at diagnosis (Dx) plus three T-ALL cell lines (CEM, 8402, HSB), one mature T cell line (Hut-102), and an EBV transformed B cell line (8432). Full length 1.6-kb α TCR transcripts are noted by dash marks, and the 8432 B cell line reveals a truncated 1.3 kb RNA γ actin hybridization is shown below.

kb Bam HI C_γ containing fragments were of approximately equal intensity in patient 3 suggesting that there was no deletion of the γ locus either. No rearrangement was seen with Sac I or PvU II digests either (data not shown). No RNA was available for evaluation of TCR gene transcription, but the T_3 molecule was absent indicating the lack of a complete T_3-Ti complex. This cell suggests that β can rearrange without preexisting γ rearrangements.

Several other leukemias demonstrated atypical combinations of TCR gene expression and cell surface antigen phenotype worth noting (Table I). T-ALL patient 17 clearly expressed 1.3 kb β TCR RNA and displayed T_3, yet lacked detectable α TCR RNA. While we cannot exclude the presence of small amounts of α RNA it is conceivable that this T cell has a T_3 receptor complex without an α-chain contribution. In contrast, T-ALL patient 11 expressed both full length α and β TCR RNA yet demonstrated the T3 molecule on only 7% of cells. Thus, this T cell may possess either a defective α or β TCR chain or alternatively a deficit in one of the various components of the T3 molecule.

ALLs germline for both TCR and Ig genes. Two ALLs lacked rearrangement of either Ig or TCR genes. Patient 5 had germline Ig heavy, κ, λ genes as well as germline γ and β TCR when assessed with three enzymes Bam HI, Eco RI, and Hind III (Fig. 5 *A*, Table I). This cell expressed cell surface 3A-1 (91%), but lacked the more definitive antigens of T cell commitment such as T_{11} or T_3. A small percentage of the leukemia cells actually expressed myeloid associated markers Mo2 (6%), LeuM1 (27%), and Leu M3 (7%) but failed to demonstrate myeloperoxidase. Patient 18 also demonstrated entirely germline configurations of Ig and TCR genes and expressed cell surface 3A-1 as well as T_{11} (Fig. 5 *B*, Table II). The presence of γ and β TCR gene rearrangement in several T-ALLs with 3A-1, but no T_{11} suggests that the acquisition of T_{11} may frequently follow γ and β TCR rearrangement (Table I). However, patient 18 indicates that this developmental sequence is not fixed as T_{11} is present in the absence of gene rearrangement. Patients 18 and 5 represent very immature leukemic cells that are uncommitted at the Ig and TCR gene level.

Lineage spillover of Ig and TCR gene rearrangements. We examined the status of TCR genes in what had been classified as pre-B cell ALL and conversely the Ig genes in leukemias categorized as T cell ALL. This comparison revealed a higher incidence of TCR rearrangement (especially γ) in pre-B cell ALL than Ig rearrangements within T cell ALL (Tables I–III). ALLs were classified as pre-B cell in type if they expressed surface B4, HLA-DR, as well as rearranged Ig heavy chain genes and lacked T cell associated antigens (Table II). A portion of these pre-B cells also displayed B1 and had progressed to κ or λ light chain rearrangement. This pre-B cell ALL group had rearranged γ TCR genes in eight of 17 cases (Table II, III). Moreover, the presence of light chain gene rearrangement did not preclude γ TCR rearrangement as pre-B ALL patients 1, 2, 4, and 17 rearranged both sets of genes. However, the overall pattern of γ TCR rearrangement in the pre-B ALLs was different than that observed in the T cell ALLs (Fig. 3 *D*). Pre-B ALLs at times rearranged only a single γ TCR allele (5/8). In contrast to the T-ALL pattern (Fig. 3 *A*) the 12.5-kb Bam HI fragment containing germline $C_\gamma 2$ is retained in the pre-B ALLs with γ rearrangements (Fig. 3 *D*). Even the pre-B cells that rearranged both γ TCR alleles retained one or both copies of the germline 12.5 kb $C_\gamma 2$. Thus, the pre-B ALLs rearrange the $C_\gamma 1$ complex frequently in contrast to the T ALLs that rarely use it and preferentially rearrange into $C_\gamma 2$. Moreover, pre-B cells with γ TCR rearrangement frequently retained germline β TCR genes (6/8) as contrasted with the generalized coordinate rearrangement of both β and γ in T-ALLs. In addition, β TCR rearrangements were noted in 3 of 17 pre-B ALLs examined including one example in which β was rearranged but γ TCR genes were germline (Tables II, III).

We wished to determine if the particularly high frequency of γ TCR rearrangement in pre-B cell ALL was peculiar to this leukemic subset of cells or reflected a high incidence of γ TCR rearrangements within the entire B cell lineage. To resolve this we examined 12 neoplasms of mature B cell phenotype as well as 14 clonal Epstein Barr Virus (EBV) transformed mature B cell lines. None of these 26 mature B cells possessed rearranged γ TCR genes (Table III). 4 of the 26 mature B cells rearranged one allele of their β TCR genes (Table III). Thus, the incidence of β TCR rearrangement in pre-B cells (3/17) versus mature B cells (4/26) was not grossly different. However, the frequency of γ TCR rearrangements in pre-B cells (8/17) was much different than in mature B cells (0/26). Therefore, there does not appear to be a generalized high rate of γ TCR rearrangement in the B cell lineage as rearrangements were not found in the mature B cell tumors. This indicates that the pre-B cell ALLs are a distinct leukemic subset and do not represent the same progenitor cells that generate the mature neoplasms.

Transcription of the α TCR locus is not lineage restricted either. Truncated 1.3-kb α messages were found in mature B cell lymphoma cell line SU-DHL-6, EBV B cell line 8432, and pre-B cell line RS (Fig. 2, Table III). In addition, even a truncated 1.0-kb β transcript was present in the Raji Burkitt B cell lymphoma cell line.

Consistent with our prior studies, Ig H chain gene rearrangement was observed in some T cell ALLs but at a low incidence (3/25) (Tables I, III). None of the T cells examined had rearranged their κ or λ light chain genes compatible with this being

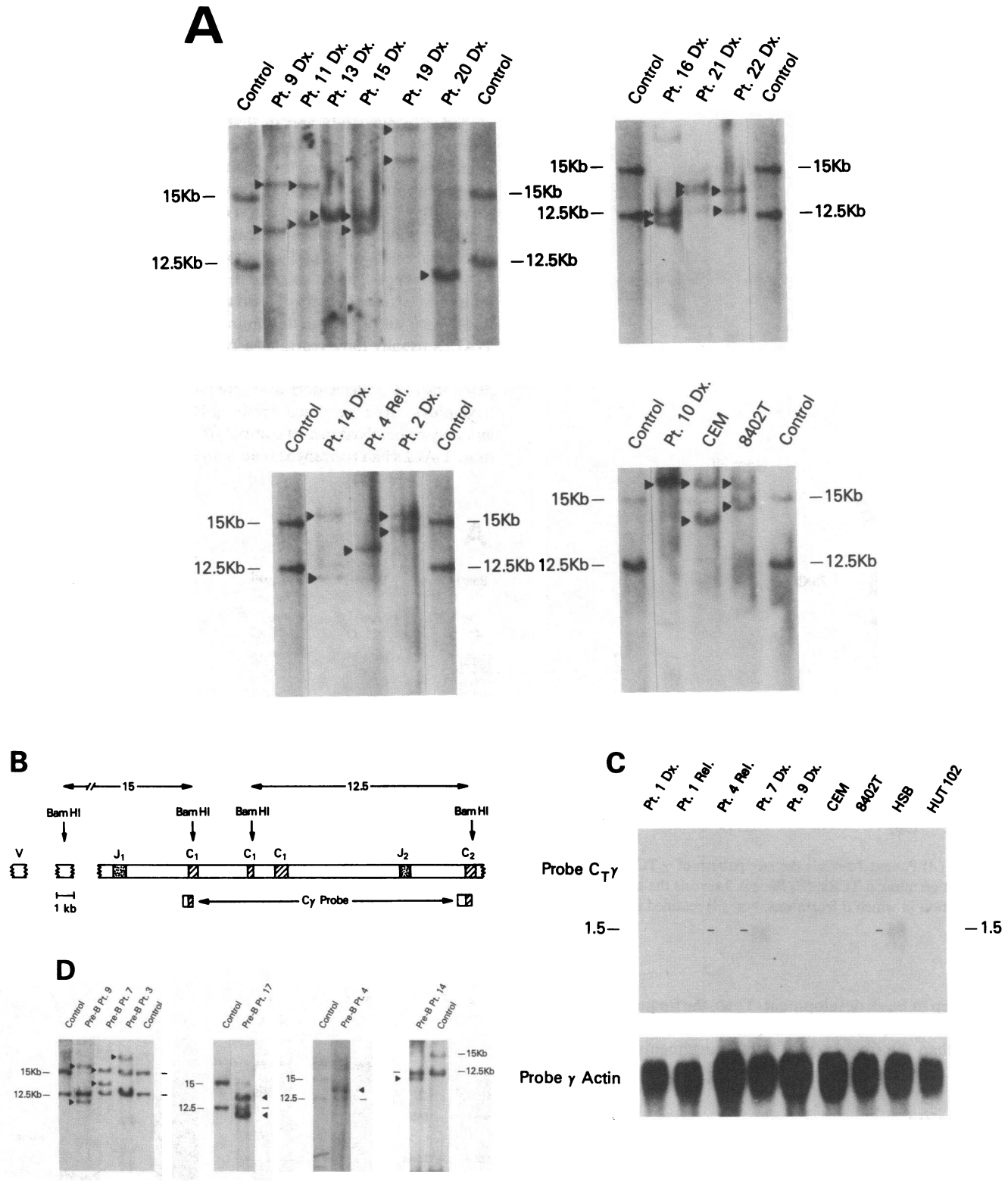

Figure 3. (*A*) γ TCR gene configuration in T-ALLs at diagnosis (Dx) or Relapse (Rel) and in two T-ALL lines. Bam HI digests probed with a Cγ probe place the $C_\gamma 1$ complex on a 15-kb fragment and $C_\gamma 2$ on a 12.5-kb fragment. Rearrangements (*arrow*) of both alleles are common. Deletion of both germline $C_\gamma 1$ and $C_\gamma 2$ bands indicates a nearly uniform usage of the $C_\gamma 2$ complex. (*B*) Schematic presentation of the human γ TCR locus and the Bam HI restriction sites. (*C*) Northern analysis of T-ALLs and T ALL cell lines plus Hut 102 for Cγ expression. Full length 1.5 kb γ TCR transcripts are indicated by the dash marks. γ-actin hybridization is shown below. (*D*) γ TCR configuration in Bam HI digested DNA from pre-B cell ALL patients. In contrast to the T-ALLs, the pre-B cells retain a germline copy of $C_\gamma 2$ (12.5 kb) when they rearrange.

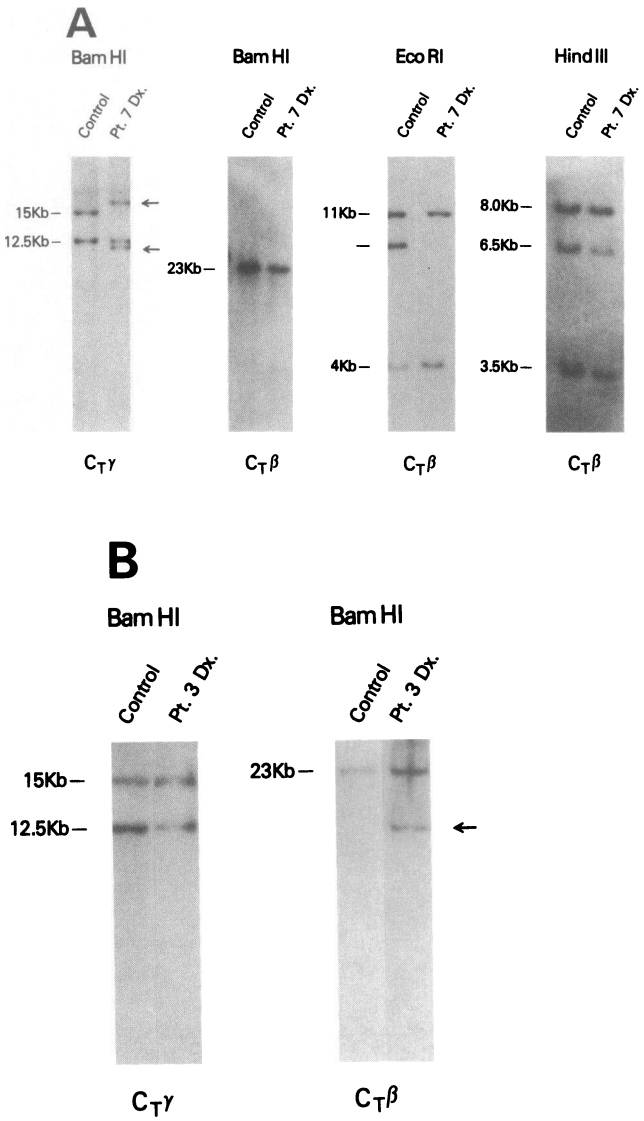

Figure 4. (*A*) Patient 7 reveals the rare pattern of γ TCR rearrangement, but germline β TCRs. (*B*) Patient 3 reveals the unusual TCR configuration in which β rearranges, but γ is retained in the germline form.

a later step in B cell development. Thus, the frequency of γ TCR rearrangement spillover into the pre-B cell ALLs was also much greater than the frequency of Ig rearrangements within T cell ALLs.

Discussion

Developmental hierarchy of TCR activation and T cell antigen expression. The determination of α, β, γ TCR gene status plus Ig gene configuration can be combined with lineage associated surface antigens to classify nearly all ALLs as pre-B or T in type. The extensive characterization of TCR genes in this study revealed that their activation is not solely restricted to the T cell lineage, just as Ig gene rearrangement is not restricted to B cells. However, the T cell ALLs did reveal a rough hierarchy of TCR activation events and cell surface antigen expression within the T lineage (Fig. 6). Only the most mature T-ALLs which had 3A-1, T_{11} and usually T_3 expressed the α TCR. The T ALLs that expressed the α TCR also expressed β TCR with one important exception (Table I). Most T-ALLs are of sufficient maturity to have rearranged both their γ and β TCR genes and these events in general precede α TCR expression. Most T-ALLs that rearranged β in our study and in that by Sangster et al. (62) also expressed it suggesting that once rearranged, the β TCR locus continues to be expressed throughout development. In contrast only occasional T-ALLs that rearranged the γ TCR actually expressed it (3/14) indicating that it may be expressed only during a narrow window of development. It is possible that this gene is vestigial by the stage of T cell development that most of these T-ALLs reflect. This is consistent with a proposed murine sequence in which γ expression precedes α, while β is expressed throughout all of thymic development (33–36). Curiously, the T-ALLs usually have rearranged both γ TCR alleles and have almost always utilized the $C_\gamma 2$ complex (Fig. 3 *A*). Interestingly, deletions of C_γ alleles were also observed. Such deletion events have previously been noted within Ig H chain or κ L chain loci and may be involved in allelic or isotypic exclusion (2, 50). While most T-ALLs had rearranged both γ and β TCR genes important

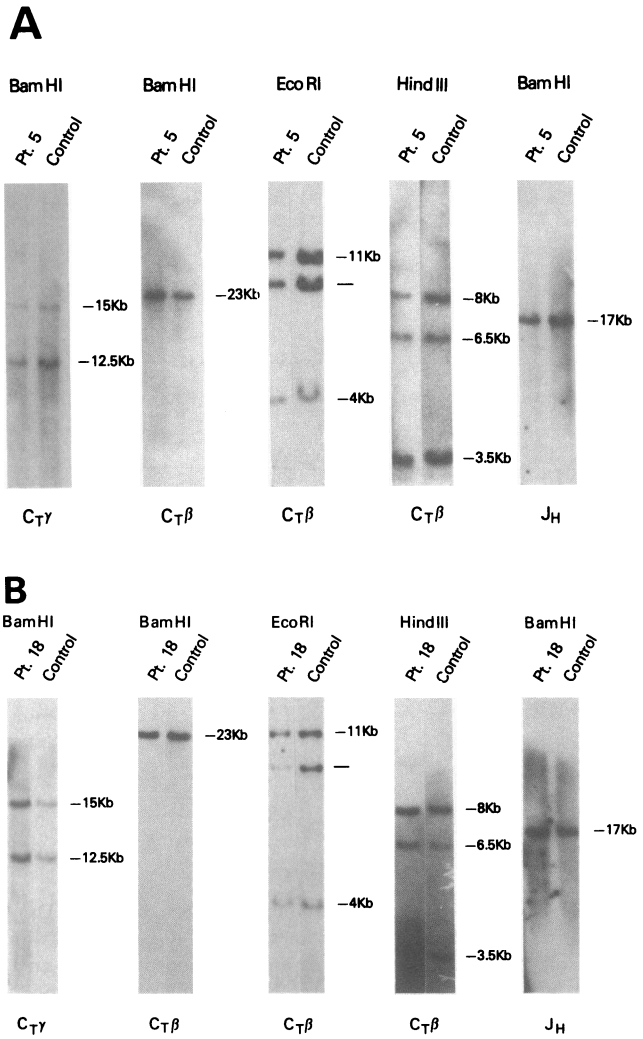

Figure 5. (*A*) Patient 5 demonstrates germline γ and β TCR genes as well as Ig H-chain genes when examined with the J_H probe. (*B*) Similarly, patient 18 is germline for γ and β TCR and Ig J_H genes.

Table II. B-Precursor ALL Patients

Patient No.	TCR Genes				Ig Genes						Phenotype*						
	$C_T\gamma$	No.	$C_T\beta$	No.	J_H	No.	C_k	No.	C_λ	No.	3A1	T11/SRBC-R	T3	B4	DR	Calla	B1
			Bam HI														
1	G		R	1	R	1			R	1	−			+	+	11	−
2‡	R/D	1/1	G		R	2	D	2	R	1	−	−	−	+	+	+	
3	R	1	G		R	2	G				−	−		+	+	+	+
4	R/D	1/1	G		R	1	R	1			−	−				+	
5	G		G		R	1	G				−	−		+	+	+	
6	G		G		R	2	G				−	−		49	+	+	
7	R	2	G		R	1	G				−	−	−	+	+	+	22
8	G		G		R	2					−	−	−	+	+	+	+
9	R	2	R	2	R	1	G				−	−	−	+	+	+	49
10	R	1	G		R	1	G				−	32	−	+	+	35	−
11	G		G		R	2	G		G		−	−	−	+	+	−	
12	G		G		R	1					18	22		45	+	−	−
13‡	G		G		R	2	R	1	G		−	−		+	+	+	
14	R/D	1/1	G		R	2					−			+	+	+	46
15	G		G		R	1	R	1			−	−	−	+	+	−	−
16	G		G		R/D	1/1	G		G		12	30	−	+	+	+	
17‡§	R	2	R	2	R	2	R/D	1/1	G		32		25	+	+	+	

* R, rearranged, D, deleted, G, germline, No., number of alleles. Data in which a (−) is < 10% of cells showing reactivity, a (+) is > 50% reactivity, and specific values are given for the 10–50% range. § Patient 17's WBC was only 7,800 and but 53% blasts at the time of this study. The 3A-1 reactivity (32%) and T3 reactivity (25%) were contributed by contaminating normal T cells. ‡ The B cell associated monoclonal antibody BA-1 was substituted for B4 in these cases.

exceptions were discovered. Examples of γ without β TCR rearrangement and also β without γ TCR rearrangement were noted here (Fig. 4 *A, B*). Similar configurations were present in the B cells bearing TCR rearrangement (Table II). These cells argue that there is no strict order for the initiation of γ and β TCR rearrangement. However, we can not exclude the possibility that unanticipated regions of the γ TCR region might exist that can not be detected at present. The earliest T cell associated antigen to appear is the 3A-1 molecule although this antigen is not completely restricted to T cells (Fig. 6) (52). The T_{11} or sheep red blood cell (sRBC) rosette receptor follows and T_3 is present following the expression of the α TCR and the presumed generation of the T_i heterodimer.

Table III. Lineage spillover

T-ALL patients and cell lines	
Rearranged J_H:	3/25
Rearranged C_k:	0/21
Rearranged C_λ:	0/23
B-Precursor ALL patients	
Rearranged TCR γ:	8/17
Rearranged 2 alleles TCR γ:	3/8
Rearranged 1 allele TCR γ:	2/8
Rearranged 1 allele TCR γ, deleted 1 allele TCR γ:	3/8
Rearranged TCR γ, but not TCR β:	6/17
Rearranged TCR β:	3/17
Rearranged TCR β, but not TCR γ:	1/17
Rearranged TCR γ and TCR β:	2/17
Mature B-cell malignancies	
Rearranged TCR γ:	0/12
Rearranged TCR β:	1/12
Rearranged 1 allele TCR β:	1/1
Clonal EBV cell lines	
Rearranged TCR γ:	0/14
Rearranged TCR β:	3/14
Rearranged 1 allele TCR β:	3/3

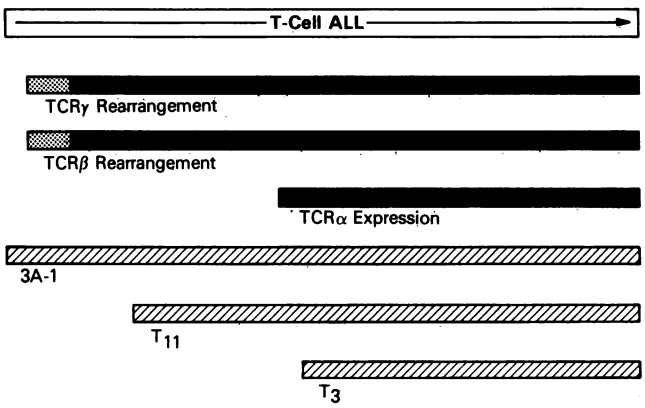

Figure 6. Proposed coordinate sequence of TCR rearrangement and expression plus cell surface antigen expression in T-ALL. 3A-1 appears to precede TCR gene rearrangement. γ and β TCRs rearrange early in development, but in no fixed order. Most T ALLs are mature enough to have rearranged both γ and β TCR and approximately one-half express the α TCR locus. The T11 molecule follows 3A-1 and T3 appears around the time of α TCR expression.

Unique T cell subsets. The exceptional T-ALL cases presented in Fig. 4 *A, B* may represent unanticipated subpopulations of T cells immortalized by a transformation event. Patient 7 expresses a unique α and γ TCR transcript and displays T_3 yet neither expresses nor even rearranges the β TCR complex. This suggests that alternative heterodimer molecules ($\alpha\gamma$, or αx, or γx) or even single chains might be complexed with the T_3 molecule within certain cells. This leukemia provides an opportunity to further define a potentially unique receptor complex that has also been proposed in nonleukemic T cell subsets (31, 32). Conversely, patient 3 rearranges β but not γ, suggesting that γ rearrangement is not a necessary first event in TCR activation.

Lineage spillover of TCR rearrangement. Only 10% of T cell ALLs in this and prior studies rearranged their Ig H chain genes. Even this small group provides occasional evidence for lineage duality. For example T-ALL patient 16 who rearranged his Ig H chain gene also had the unusual presence of HLA-DR (usually seen on pre-B ALL). Provocatively, he failed to express any of the TCR loci despite rearrangements (Table I). Perhaps sharing events of B and T cell pathways is reflected by this cell's failure to progress further in either B or T cell maturation.

ALLs that we would have categorized as being pre-B cell in type showed a surprising amount of γ TCR gene rearrangement. While $\sim$ 20% of pre-B ALLs rearranged their β-chain genes, roughly 45% rearranged the γ TCR (Table II, Fig. 3 *D*). Several lines of evidence argue that these cells are predominately B cell lineage committed rather than being mis-classified early T cells. All of these cells bore B cell surface antigens that are rarely or never seen in T-ALLs including HLA-DR, B_4 and often B_1. They had uniformly rearranged their H chain genes and some had progressed to L chain genes. Moreover, even the apparently out of context γ TCR rearrangement displayed a different pattern in these pre-B cells than it did in the T-ALLs. The γ TCR rearrangements in pre-B cells were more often on only one allele and frequently utilized $C_\gamma 1$, whereas the T ALLs preferentially rearranged into the $C_\gamma 2$ complex. Thus, by many criteria this group of cells differs from the T cell set and more closely resembles the other pre-B cells.

Strikingly however, this high incidence of γ TCR rearrangement was not witnessed in the mature B cell neoplasms and cell lines. This group of pre-B ALLs thus do not represent the progenitor cells that give rise to these more mature cells. Once again, there was a dissociation of γ and β TCR rearrangement in pre- and mature B cells arguing against a srict order of locus accessibility and rearrangement in TCR activation. It is important that the high frequency of γ TCR rearrangement argues that the pre-B ALLs represent a rather distinct population of cells. Several potential explanations for this unanticipated event exist. The pre-B ALLs with rearranged γ TCR might represent a unique subpopulation of cells that are made evident only by malignant transformation and subsequent clonal outgrowth. It is possible that the normal counterparts of these leukemias are precluded from developing further into either B or T cell pathways. They lack the Ig production of a B cell yet their peculiar γ TCR rearrangement pattern might preclude further effective T cell differentiation. Such cells might be confused with respect to their lineage commitment. Alternatively, it is conceivable that the γ TCR rearrangements are a secondary event following transformation. Perhaps these ALLs reflect early lymphoid progenitors lacking either complete Ig or TCR rearrangements and because of this a putative common recombinase would remain active. By this proposal the γ TCR locus might be particularly vulnerable to further recombination. In this context it would not be surprising if the specific pattern of γ rearrangement were different in pre-B or uncommitted cells than in a truly committed T cell.

Standards of lineage commitment. Considerable spillover of Ig H chain rearrangement within bona fide mature T cells and TCR activation within bona fide mature B cells have been noted in this and previous studies (2, 14, 63, 64). Some of these events may be even more pronounced earlier in differentiation. This lack of total lineage fidelity of these genetic events necessitates some caution in solely using these markers to determine a lineage designation. Ig L chain rearrangement appears to be a later event in development and to date has been restricted to B cell development. However, in the more immature pre-B cells with only H-chain rearrangement the presence of the B cell associated antigens B_4 and B_1 as well as HLA-DR are important. The lack of TCR activation also favors a B cell assignment, but some pre-B cells will rearrange γ or β and even express α TCR genes.

One of the strongest criteria for T cell commitment in ALLs is the lack of Ig H and L chain rearrangement and the lack of the B cell surface antigens. The exclusive presence of rearrangements of TCRs is also strong evidence favoring a T lineage commitment, although the pattern of α, β, and γ TCR rearrangement and expression can be varied. The 3A-1 antigen is seen on early T cells, but is not fully lineage restricted, whereas the T_{11} and T_3 molecules appear later in development and are more definitive.

Early lymphoid progenitors within ALL. Cells from two T ALL patients lacked both Ig and TCR rearrangements (patient 5 and 18). One of these cells possessed only the 3A-1 molecule and also displayed some myeloid antigens on a subpopulation of the leukemic cells. It thus appeared to be rather lineage indeterminant by several criteria. The other leukemia displayed T_{11} and 3A-1 suggesting a T cell identity but had not genetically committed its TCR loci. These cells provide an opportunity to dissect even earlier stages of commitment in putative lymphoid progenitors. The extensive characterization of Ig loci, TCR loci, and surface antigens has helped define the developmental state of individual T and B cells, revealed unanticipated TCR rearrangement events and clonal expansions of unique and potentially rare lymphocyte subpopulations.

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