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Immunoglobulin and T-cell receptor gene rearrangement and expression in human lymphoid leukemia cells at different stages of maturation

(clonality/lineage/T-lymphocyte differentiation)

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ABSTRACT **The use of probes to genes (*IG* and *TCRB*) encoding immunoglobulins (IG) and the β chain of the T-cell antigen receptor (TCRB), respectively, have become a sensitive means to assess clonality and lineage in lymphoid malignancies. It has become apparent that some individual cases show rearrangements of both *IG* and *TCRB* genes. In an attempt to more accurately define cell lineage we have analyzed cells from patients with B- or T-cell leukemia ($n = 26$) at various stages of maturation with probes to two additional TCR genes, *TCRG* and *TCRA* (encoding the TCR γ and α chains, respectively), as well as the *IG* heavy chain joining region (*IGHJ*) and *TCRB* genes. On Southern blot analysis, the mature T-cell leukemia cells studied had rearranged *TCRG* and *TCRB* while *IGHJ* remained as in the germ line. The mature B-cell leukemia cells studied had rearranged *IGHJ* with germ-line *TCRG* and *TCRB*. These data suggest that, in the majority of more mature leukemias, cells have rearranged *IG* or *TCR* genes but not both. In contrast, cells from five of nine precursor B-cell leukemia patients and cell lines from one of four precursor T-cell leukemia patients had rearranged both *IGHJ* and *TCR* genes. TCRG and TCRB mRNAs were expressed in the cells of precursor T- but not B-cell leukemia patients studied. The spectrum of leukemia cells studied within the T-cell series permitted an assessment of the order of *TCR* gene rearrangements. Two of 13 patients had cells with germ-line *TCRG* and *TCRB*, 2 patients had cells with rearranged *TCRG* alone, and the remainder had cells with rearranged *TCRG* and *TCRB*. TCRG and TCRB mRNAs were expressed in precursor T-cell leukemia cells, whereas *TCRB* and *TCRA* were expressed in mature T-cell leukemia cells. These results parallel observations from mouse studies on gene expression and support the view of a hierarchy of *TCR* gene rearrangements in T-lymphocyte ontogeny. *TCRG* genes are rearranged first, subsequently *TCRB* genes are rearranged, followed by *TCRA* gene activation.**

The T-cell antigen receptor (TCR) is a 90-kDa heterodimer consisting of 40- to 50-kDa α and β subunits (TCRA and TCRB, respectively). Similar to the immunoglobulin (*IG*) genes, the genes encoding the subunits undergo somatic rearrangements in the course of lymphocyte ontogeny (see ref. 1 for review). The human *TCRB* gene complex in its germ-line form is composed of discontinuous gene subsegments consisting of multiple variable regions (V_β) with duplicate sets of diversity (D_β), joining (J_β), and constant (C_β) gene segments (Fig. 1*B*). At some point during the differentiation of a pluripotent stem cell into a mature T cell, a process of DNA rearrangement juxtaposes a D_β with a J_β segment and then a V_β region with this D_β–J_β junction to assemble the complete variable region gene and permit transcription of mRNA for the complete TCRB peptide. The DNA recombinations generate changes in the location of restriction endonuclease sites that can be used in Southern blot analysis to distinguish the rearranged from the germ-line form of this gene. The *TCRA* gene complex consists of at least 13 families of V_α genes and perhaps 50 J_α gene segments in a tandem array present in a greater than 60-kilobase (kb) region on the 5′ side of a single C_α gene. Detecting rearrangement of the *TCRA* locus using C_α probes has been difficult because the restriction enzymes used have sites of action between C_α and the 5′ J_α genes and DNA recombinations would not give a new band on Southern blot analysis. A third gene, *TCRG*, has been identified in T cells that has many properties in common with *TCRA* and *TCRB* genes including assembly from gene segments resembling *V*, *J*, and *C* regions, rearrangement, and expression in T cells (2–4). In addition to multiple variable region genes there are at least two *J* and two *C* region segments (Fig. 1*C*) (5, 6).

Over the last five years it has been possible to determine clonality, lineage, and stage of maturation of lymphoid malignancies by analyzing the arrangement of genes encoding *IG* (7–9) and *TCRB* (10). Furthermore, an analysis of *IG* gene rearrangements in precursor B-cell leukemia cells has revealed a hierarchy of *IG* gene rearrangements in which heavy chain genes precede light chain genes and κ light chain genes precede λ light chain genes (7, 11). However, while this type of analysis has permitted the assignment of a population of patients with the same type of leukemia to a specific lineage, ambiguity can still exist in individual cases at the gene level. For example, approximately 30% of patients with acute lymphocytic leukemia (ALL) that have cells that lack T-cell markers and surface IG rearrange both *IG* and *TCRB* genes (12). In an attempt to clarify such cases of ambiguous lineage, we have analyzed the arrangement of *IG* and *TCR* genes in mature and precursor B- and T-cell leukemia cells at various stages of maturation. *IG*, *TCRG*, and *TCRB* gene rearrangements were studied as well as expression of TCRG, TCRB, and TCRA mRNAs. Leukemia cells from several of the patients exhibited a more immature phenotype than those in previous studies. The spectrum of leukemias studied provid-

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Abbreviations: ALL, acute lymphocytic leukemia; ATL, adult T-cell leukemia; C, constant; CLL, chronic lymphocytic leukemia; D, diversity; J, joining; TCR, T-cell receptor; V, variable; α, α subunit of TCR; β, β subunit of TCR; γ, γ subunit of TCR; LC, leukemia cells; kb, kilobase(s).

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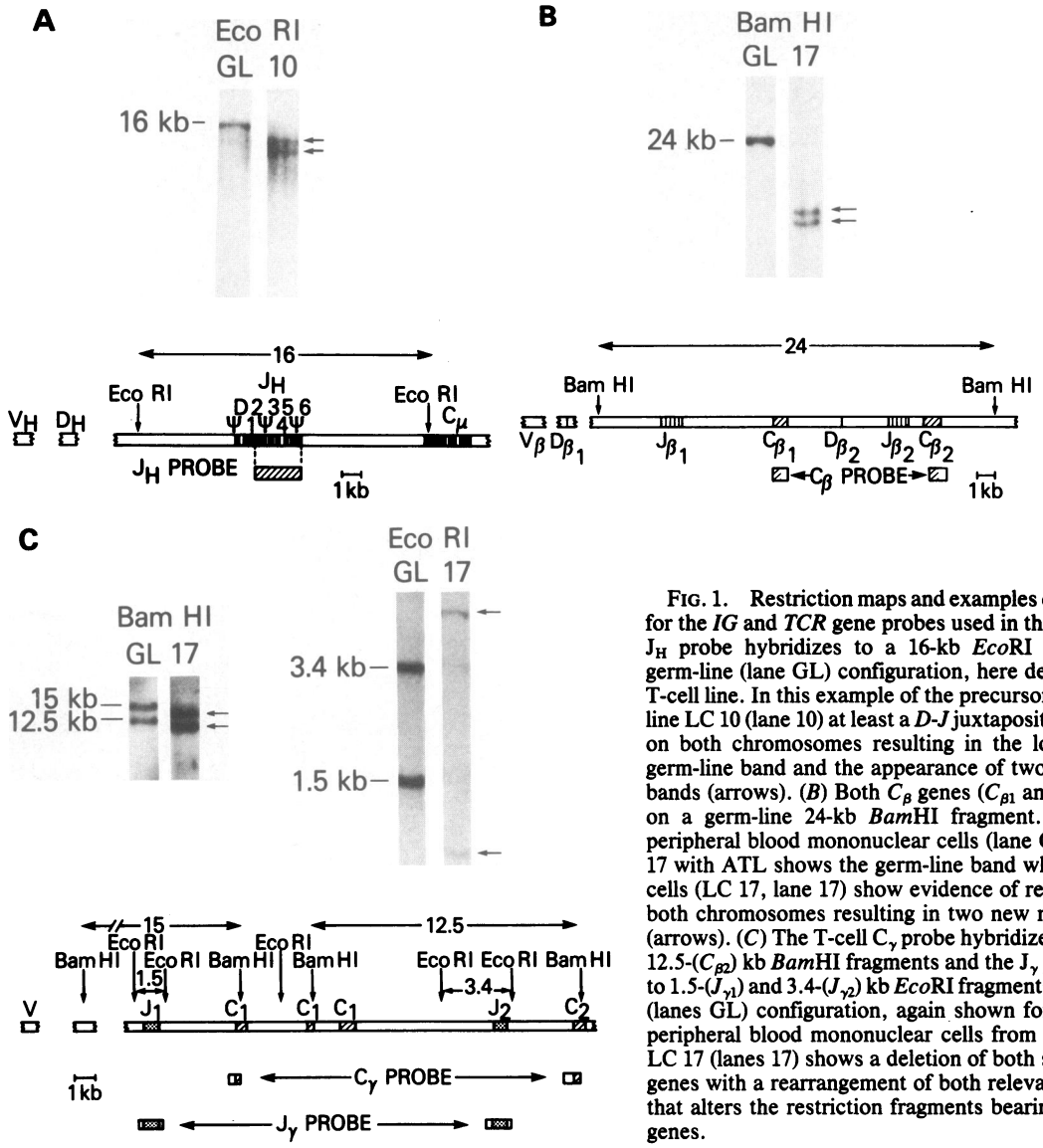

FIG. 1. Restriction maps and examples of Southern blots for the *IG* and *TCR* gene probes used in this study. (*A*) The J_H probe hybridizes to a 16-kb *Eco*RI fragment in the germ-line (lane GL) configuration, here demonstrated by a T-cell line. In this example of the precursor B-cell leukemia line LC 10 (lane 10) at least a *D-J* juxtaposition has occurred on both chromosomes resulting in the loss of the 16-kb germ-line band and the appearance of two new rearranged bands (arrows). (*B*) Both C_β genes ($C_{\beta 1}$ and $C_{\beta 2}$) are found on a germ-line 24-kb *Bam*HI fragment. T-cell depleted peripheral blood mononuclear cells (lane GL) from patient 17 with ATL shows the germ-line band while the leukemia cells (LC 17, lane 17) show evidence of rearrangements on both chromosomes resulting in two new rearranged bands (arrows). (*C*) The T-cell C_γ probe hybridizes to 15-($C_{\beta 1}$) and 12.5-($C_{\beta 2}$) kb *Bam*HI fragments and the J_γ probe hybridizes to 1.5-($J_{\gamma 1}$) and 3.4-($J_{\gamma 2}$) kb *Eco*RI fragments in the germ-line (lanes GL) configuration, again shown for T-cell depleted peripheral blood mononuclear cells from patient 17. Here LC 17 (lanes 17) shows a deletion of both sets of *J1* and *C1* genes with a rearrangement of both relevant chromosomes that alters the restriction fragments bearing the *J2* and *C2* genes.

ed data consistent with a hierarchy of *TCR* gene rearrangement and expression comparable to the *IG* gene rearrangement observed in leukemias of the B-cell series. A preliminary report of this work has been presented.[‖]

MATERIALS AND METHODS

Cells. DNA and RNA were extracted from cell lines or from cell suspensions of peripheral blood mononuclear cells from patients grouped according to the following characteristics. Leukemia cells 1–4 (LCs 1–4), from patients 1–4, respectively, with chronic lymphocytic leukemia (CLL), expressed surface IG and had clonal rearrangements of both heavy and light chain *IG* genes. These patients were considered to have mature B-cell leukemias. LCs 5–9, from patients 5–9, respectively, with ALL, had leukemia cells that reacted with the monoclonal antibody B4, lacked surface IG, failed to react with any T-cell specific monoclonal antibodies, and showed rearrangement of the *IGHJ* gene. These patients were considered to have precursor B-cell leukemias. LCs 10–13 and 18–21 represent precursor B- and T-cell lines, respectively, whose phenotypes and *IG* gene patterns have been described (8). There were cells from four patients (LCs 14–17) with adult T-cell leukemia (ATL) based on described criteria (13). These patients were considered to have mature T-cell leukemias. LCs 22–26, considered to represent either stem- or precursor T-cell leukemias (14), expressed the 3A1 antigen (CD-7) (15, 16) but lacked T3, T4, T6, and T8 antigens. LCs 22–26 were negative for BA1, B1, or B2 expression and, with the exception of LC 23, did not express the MO1 antigen. LCs 22 and 26 expressed the T11 antigen (CD-2). In addition, when placed in culture, LCs 22, 24, and 26 had the ability to spontaneously differentiate along myeloid, erythroid, and megakaryocytoid lineages, respectively (ref. 17; J.K., M.S.H., and B.F.H., unpublished data).

Flow Cytometric Analysis. The source of the monoclonal antibodies and the methods used for the phenotypic determinations were performed as described (13).

Southern Blot Analysis. DNA extraction, gel electrophoresis, transfer, and hybridization were performed as described (8, 9). The probes used were as follows: J_H, a 2.4-kb germ-line *Sau*3A fragment that recognizes the *IGHJ* locus (Fig. 1*A*) (18); C_B, a 700-base-pair *Eco*RI fragment containing a mouse C_β region that recognizes both human C_β genes (Fig. 1*B*) (13); T_α, a 1.1-kb fragment containing *V*, *J*, and *C* sequences (19) (a kind gift from Tak Mak); TC_γ, a 300-base-pair *Pst* I–*Bam*HI fragment that recognizes both C_γ genes (Fig. 1*C*) (4); and TJ_γ,

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a 1.0-kb *Eco*RI–*Hin*dIII fragment that recognizes both J_γ genes (Fig. 1*C*) (6).

RNA Gel Blot Analysis. RNA was extracted by the guanidinium isothiocyanate technique (20). Total (20 μg) and poly(A)-selected (10 μg) mRNA was denatured in 50% (vol/vol) formamide at 65°C and electrophoresed through 1% agarose with 6.6% (vol/vol) formaldehyde in Mops buffer (20). The RNA was transferred to a nylon matrix (Zeta-Probe; Bio-Rad). Nylon blots were used for repeated hybridizations by washing in 0.1% SSC/0.1% $NaDodSO_4$ at 95°C for 20 min (SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

RESULTS

***IG* and *TCR* Gene Rearrangements in B- and Precursor B-Cell Leukemia Cells.** The normal germ-line arrangements of *IGHJ*, *TCRG*, and *TCRB* genes for the various ^{32}P-labeled probes and restriction enzymes were defined by examining DNA from circulating leukocytes from healthy persons. The J_H probe detects a germ-line 16-kb *Eco*RI fragment (18) (Fig. 1*A*). The C_β probe hybridizes to both C_β gene segments on a single 24-kb *Bam*HI fragment (13) (Fig. 1*B*). The C_γ genes consist of two closely related gene segments found on 15-(C1) and 12.5-(C2) kb *Bam*HI fragments (6) (Fig. 1*C*). The J_γ probe hybridizes with both J_γ genes present on 3.4-(J2) and 1.5-(J1) kb *Eco*RI fragments (Fig. 1*C*) (6).

The four patients with B-cell CLL studied had germ-line *TCRG* and *TCRB* patterns (Table 1). However, in studies including our own with larger sample sizes (12, 13), about 10% of mature B-cell leukemia cells were shown to have *TCRB* rearrangements. *IGHJ* was rearranged in all cases. Leukemia cells or cell lines from patients with precursor B-cell leukemia had rearranged *IGHJ* genes in all cases, however, *TCRG* or *TCRB* genes were also rearranged in five of nine cases studied. One contained a rearrangement of *TCRG* alone, four had rearranged both *TCRG* and *TCRB*, whereas the remaining four had retained their *TCRG* and *TCRB* genes in the germ-line configurations (Table 1). There were no cells studied with an isolated *TCRB* rearrangement.

***IG* and *TCR* Gene Rearrangements in T-, Precursor T-Cell, and Stem-Cell Leukemia Cells.** Leukemia cells from the four patients with ATL (Table 2) had a germ-line *IGHJ* pattern. In all cases there was rearrangement of *TCRG* and *TCRB* genes. Likewise, all precursor T-cell leukemia cell lines rearranged *TCRG* and *TCRB* genes (Table 2). As reported (8) the precursor T-cell line HSB-2 has rearranged *IGHJ*. A germ-line *IGHJ* pattern was present in all stem and precursor T-cell leukemia cells (Table 2). Cells from two patients (LCs 24 and 26) were germ-line for *TCRG* and *TCRB*, cells from two patients (LCs 23 and 25) had rearranged *TCRG* alone while cells from one patient (LC 22) had rearranged *TCRG* and *TCRB*. Again, there were no examples of an isolated *TCRB* rearrangement. DNA from LCs 23–26, digested with *Eco*RI and *Hin*dIII and hybridized with the C_β probe, likewise showed a germ-line pattern (data not shown).

Table 1. B-cell leukemia cells

LC	Gene configuration*			mRNA expression†		
	IG	*TCRB*	*TCRG*	TCRG	TCRB	TCRA
Chronic lymphocytic leukemia						
1	RA	GL	GL	NA	NA	NA
2	RA	GL	GL	NA	NA	NA
3	RA	GL	GL	NA	NA	NA
4	RA	GL	GL	NA	NA	NA
Precursor B-cell ALL						
5	RA	RA	RA	NA	NA	NA
6	RA	RA	RA	NA	NA	NA
7	RA	GL	GL	NA	NA	NA
8	RA	GL	RA	NA	NA	NA
9	RA	RA	RA	NA	NA	NA
Precursor B-cell lines						
10 (Reh)	RA	RA	RA	–	–	–
11 (Nalm 6)	RA	GL	GL	–	–	1.6
12 (Nall 1)	RA	GL	GL	–	–	1.6,1.3
13 (Nalm 1)	RA	GL	GL	–	–	–

RA, rearranged; GL, germline; NA, not available.
*Gene patterns were determined with J_H, C_β, C_γ, or J_γ probes.
†A minus (−) sign indicates absence of specific mRNA; the number indicates the size of the mRNA species detected in kb.

Table 2. Stem and T-cell leukemia cells

LC	Gene configuration			mRNA expression		
	IG	*TCRB*	*TCRG*	TCRG	TCRB	TCRA
Adult T-cell leukemia						
14	GL	RA	RA	1.7	1.3,1.0	1.6
15	GL	RA	RA	–	1.3	1.6
16	GL	RA	RA	–	1.3,1.0	1.6
17	GL	RA	RA	–	1.3	1.6
Precursor T-cell lines						
18 (Molt 4)	GL	RA	RA	–	1.3	–
19 (CEM)	GL	RA	RA	1.7	1.3	–
20 (HSB-2)	RA	RA	RA	1.7	1.3	–
21 (8402T)	GL	RA	RA	1.7	1.3	–
Stem and precursor T-cell leukemia cells						
22	GL	RA	RA	1.7	1.3,1.0	1.6,1.3
23	GL	GL	RA	1.7	1.0	–
24	GL	GL	GL	–	–	–
25	GL	GL	RA	1.7	–	1.6
26	GL	GL	GL	NA	NA	NA

Data are expressed as in Table 1.

Expression of TCR Genes in B- and T-Cell Leukemia Cells. Because of the difficulty in accurately determining *TCRA* gene rearrangement, we have attempted to identify activation of the *TCRA* gene complex by looking for mRNA expression of this gene. Mature TCRA mRNA encoding the complete *TCRA* peptide is 1.6 kb, however, smaller (1.3 kb), presumably immature transcripts have been described (19). Mature TCRB mRNA is 1.3 kb while an immature 1.0 kb mRNA transcript from *D-J* junctions that does not involve the rearrangement of the V_β gene can be detected (21, 22). In murine thymocytes and cytotoxic T-lymphocyte clones mRNA of ≈1.7 kb containing C_γ sequences has been detected (2, 32).

TCRG and TCRB mRNA was not detected in any of the precursor B-cell lines studied (Table 1). *TCRA* was expressed in LCs 11 and 12, where *TCRG* and *TCRB* genes were in germ-line form. Mature T-cell leukemia cells (LCs 14–17) all expressed *TCRB* and *TCRA* while *TCRG* expression was detected in one case (Table 2). None of the precursor T-cell lines (LCs 18–21) expressed *TCRA*. However, all expressed *TCRB* and with one exception (LC 18) expressed *TCRG* as well (Table 2).

Expression in stem or precursor T-cell leukemia cells (LCs 22–26) was more variable. LC 24, which showed a germ-line pattern for *TCRG* and *TCRB* genes, lacked expression of any T-cell receptor specific mRNA. Two cases (LCs 23 and 25) that expressed *TCRG* had different patterns of *TCRB* and *TCRA* expression. LC 23 had a germ-line *TCRB* pattern on Southern analysis and expressed the immature TCRB message (1.0 kb) that lacks V_β sequences. LC 25 was likewise germ line for *TCRB* but expressed TCRA mRNA. LC 22 rearranged *TCRG* and *TCRB* genes and expressed mRNA for all three T-cell receptor genes.

As a quantitative control for the presence of mRNA all samples showed a band of equal intensity when hybridized

with a cDNA probe for actin [pHF$_\gamma$A-1, a kind gift of Larry Kedes (23), data not shown].

DISCUSSION

In an attempt to define lineage in lymphoid malignancies we have analyzed *IG, TCRG,* and *TCRB* gene rearrangements in lymphoid leukemia cells at different stages of maturation. The T-cell malignancies expressing a mature phenotype studied here (e.g., ATL, Table 2) as well as the majority reported (13) show a germ-line pattern of *IGHJ* and in all cases a rearrangement of *TCRG* and *TCRB* genes. *IG* light chain gene rearrangements have not been noted in T-cell leukemia cells to date (9, 24). All mature B-cell leukemia cells analyzed here (e.g., CLL, Table 1) showed rearranged *IGHJ* and germ-line T-cell receptor genes. However, previous studies have shown ≈10% of cells from B-cell CLL patients had rearranged *TCRB* (13, 24). Furthermore, all mature B-cell leukemia cells show *IG* light chain gene rearrangements. These data suggest that when *IG* light and heavy chain gene as well as T-cell receptor probes are utilized the more mature leukemia cells show an unambiguous pattern of *IG* or *TCR* gene rearrangements.

Patients with leukemias of immature lymphocytes have cells that show a greater tendency to rearrange both *IG* and *TCR* genes. Cells from four of five patients with precursor B-cell leukemias, one of four precursor B-cell leukemia cell lines (Table 1), and one of four precursor T-cell leukemia cell lines (Table 2) manifested such dual rearrangements. Previous studies have shown (10, 13, 24) that 10% of patients with T-cell ALL and 30% of patients with non-B-, non-T-cell ALL (12) had cells with rearranged *IG* and *TCRB* genes. The greater frequency of dual rearrangements in leukemias of the precursor B-cell series is in agreement with previous studies (24). Since bands reflecting rearrangement are presumably not lost as B cells mature, the immature leukemia cells may present patterns that are not on the common pathway of normal B-cell maturation. One possible explanation for the high incidence of *TCRG* and *TCRB* gene rearrangement in precursor B-cell leukemias is that they lack the signals present in more mature cells that terminate gene rearrangements. In this view, activation of both B- and T-cell genes within the same cell may be explained in part by the use of the same recombinational mechanism by both *IG* and *TCR* genes (25–27). Recombinases responsible for *IG* and *TCR* gene rearrangements may remain active until an effective "stop signal" (e.g., mature *IG* or *TCR* molecules) is produced. Such a situation exists in B cells where additional *V* to *D-J* rearrangements are usually prevented once an effective *V-D-J* rearrangement leading to the production of an IG molecule occurs (28, 29). Thus if a precursor B cell has a productive heavy chain mRNA produced as a result of an early recombinational event, recombinases may be suppressed or open chromatin sites closed, and no further rearrangements (e.g., of T-cell genes) can occur. A transforming event after this stage would lead to an unambiguous B-cell malignancy. However, if a productive mRNA leading to the expression of *IG* is not produced, recombinases may remain active, genes of both the T- and B-cell series may be activated, and a transforming event at this stage could lead to the clonal expansion of a population of cells with dual rearrangements.

All leukemias in which *TCRB* is rearranged likewise rearranged *TCRG*, therefore, studies of *TCRG* rearrangement will not resolve the question of lineage in ambiguous cases of B- and T-cell malignancies. However, determination of TCRG and TCRB mRNA expression may be helpful. For example, the precursor B-cell precursor line Reh (LC 10) showed rearrangement of *IGHJ, TCRG,* and *TCRB*, but lacked expression of TCRG and TCRB mRNA. In contrast, the precursor T-cell cell line HSB-2 (LC 20) likewise rearranged all three genes and expressed TCRG and TCRB mRNA. One drawback in relying on mRNA for classification is that contamination of an RNA preparation with RNA from a minority normal T-cell population with high levels of expression may yield misleading results.

TCRA mRNA expression was not helpful in discriminating between cases of ambiguous lineage. As expected, it was present in mature T-cell leukemia cells (Table 2), but could also be detected in two precursor B-cell leukemia cell lines (LCs 11 and 12), and in LC 25 representing an early precursor T-cell leukemia. In addition, the lymphoblastoid B-cell partners to HSB-2 and 8402T expressed a 1.3-kb TCRA message (data not shown). The presence of TCRA mRNA expression in lymphoid cells must be interpreted with caution as situations exist where expression does not correlate with rearrangement. Various size J_α-C_α transcripts lacking translation initiation codons can be found in B cells (30). Probes to the J_α region have been developed (31) that will allow a more exact association between rearrangement and expression.

The use of DNA probes to detect clonality has several valuable clinical uses. Besides its obvious value as an adjunct to cancer diagnosis, it can be used to monitor the effect of therapy and as an early indicator of relapse (13). Analysis of leukemia cells can likewise lead to a better understanding of events that normally occur in the course of lymphocyte ontogeny. For example, by examining the spectrum of leukemias of the T-cell series described here, from stem cells or very early T-cell precursors bearing only the T-cell antigen defined by the 3A1 monoclonal antibody to mature T-cell leukemias, we provide evidence for a hierarchy of rearrangement of *TCR* genes. Two of 13 LCs had germ-line *TCRG* and *TCRB* (LCs 24 and 26), LCs 23 and 25 rearranged *TCRG* alone, and the remainder rearranged both *TCRG* and *TCRB*. With one exception (LC 18), precursor T-cell leukemia lines expressed both TCRG and TCRB mRNA while mature T-cell leukemia cells expressed TCRB and TCRA mRNA (LC 14 also expressed TCRG mRNA). Of the nine precursor B-cell leukemia cells studied, one rearranged *TCRG* alone (LC 8) while four rearranged both *TCRG* and *TCRB*. TCRA mRNA detected in the setting of germ-line *TCRB* genes (as in several B-cell lines) may represent truncated messages and not *V-J* rearrangements. The present data on human T-cell leukemia lineage parallels the observations in thymocytes of fetal mice (32) and observations with leukemia T-cell lines (31) and supports the view that the *TCRG* gene is rearranged first, followed by the *TCRB* gene. This in turn is followed by *TCRA* gene activation. Other investigators have likewise found that activation of *TCRB* genes precedes *TCRA* genes in thymic ontogeny (33–35). Thus as in B cells there is an apparent

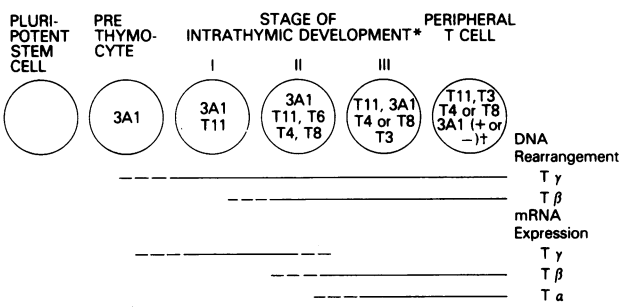

FIG. 2. *TCR* gene rearrangements and expression in relation to the coordinate sequence of cell surface antigen expression. T_γ, TCRG; T_β, TCRB; T_α, TCRA.

*The phenotypes of the leukemia T cells studied were determined and classified according to the convention of Reinherz *et al.* (36), however, we have also included 3A1, an early T-cell lineage differentiation antigen (37, 38).

†3A1 expression is variable in normal peripheral T cells. It is expressed on OKT8 cells but is lacking from a subset of OKT4 cells, Sezary cells (16, 39), and most ATL cells.

hierarchy of receptor gene rearrangement. Accompanying this is a coordinate sequence of cell surface antigen expression in these leukemia cells (Fig. 2). The earliest recognizable T-cell precursors that rearrange only the *TCRG* gene express the 3A1 antigen alone. Cells subsequently rearrange the *TCRB* gene and begin to express the antigen identified by the T11 monoclonal antibody. The mature T-cell leukemia cells express T3, T4, or T8 peptides, with rare exception no longer express 3A1, begin to express *TCRA* gene mRNA, and, for the leukemias studied here, no longer express measurable quantities of TCRG message (except LC 14). These correlations of cell surface antigen expression and T-cell receptor gene rearrangements and expression provide further evidence that the lymphocytic leukemia cells are cells at distinct stages of T-cell development.

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