Immunglobulin 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 TCRα (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 Iγ 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 TCRB 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 TCRα 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 TCRα 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 (Vβ) with duplicate sets of diversity (Dβ), joining (Jβ), and constant (Cβ) gene segments (Fig. 1B). At some point during the differentiation of a pluripotent stem cell into a mature T cell, a process of DNA rearrangement juxtaposes a Dβ with a Jβ segment and then a Vβ region with this Dβ-Jβ 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 TCRB gene complex consists of at least 13 families of Vβ genes and perhaps 50 Jβ gene segments in a tandem array present in a greater than 60-kilobase (kb) region on the 5′ side of a single Cβ gene. Detecting rearrangement of the TCRB locus using Cβ probes has been difficult because the restriction enzymes used have sites of action between Cβ and the Jβ genes and DNA recombinations would not give a new band on Southern blot analysis. A third gene, TCRα, has been identified in T cells that has many properties in common with TCRα 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 Jα and two C region segments (Fig. 1C) (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 λ 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) 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, TCRB, and TCRB gene rearrangements were studied as well as expression of TCRG, TCRB, and TCRB mRNAs. Leukemia cells from several of the patients exhibited a more immature phenotype than those in previous studies. The spectrum of leukemias studied provid-

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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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.11

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 IGHI 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 B1A, 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: JH, a 2.4-kb germ-line Sau3A fragment that recognizes the IGHI locus (Fig. 1A) (18); C9, a 700-base-pair EcoRI fragment containing a mouse C9 region that recognizes both human C9 genes (Fig. 1B) (13); Tα, a 1.1-kb fragment containing V, J, and C sequences (19) (a kind gift from Tak Mak); TC2, a 300-base-pair Pst I–BamHI fragment that recognizes both C2 genes (Fig. 1C) (4); and TJ,
a 1.0-kb EcoRI–HindIII fragment that recognizes both J<sub>G</sub> genes (Fig. 1C) (6).

**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 32P-labeled probes and restriction enzymes were defined by examining DNA from circulating leukocytes from healthy persons. The J<sub>H</sub> probe detects a germ-line 16-kb EcoRI fragment (18) (Fig. 1A). The C<sub>G</sub> probe hybridizes to both C<sub>G</sub> genes segments on a single 24-kb BamHI fragment (13) (Fig. 1B). The C<sub>G</sub> genes consist of two closely related gene segments found on 15-(C1) and 12.5- (C2) kb BamHI fragments (6) (Fig. 1C). The J<sub>G</sub> probe hybridizes with both J<sub>G</sub> genes present on 3.4- (J2) and 1.5- (J1) kb EcoRI fragments (Fig. 1C) (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 TCRB 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. Stem and T-cell leukemia cells**

<table>
<thead>
<tr>
<th>Gene configuration</th>
<th>mRNA expression</th>
</tr>
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<tbody>
<tr>
<td>LC</td>
<td>TCRG</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>RA</td>
</tr>
<tr>
<td>Precursor B-cell ALL</td>
<td>RA</td>
</tr>
<tr>
<td>Precursor B-cell lines</td>
<td>RA</td>
</tr>
<tr>
<td>10 (Reh)</td>
<td>RA</td>
</tr>
<tr>
<td>11 (Nalm 6)</td>
<td>RA</td>
</tr>
<tr>
<td>12 (Nall 1)</td>
<td>RA</td>
</tr>
<tr>
<td>13 (Nalm 1)</td>
<td>RA</td>
</tr>
</tbody>
</table>

RA, rearranged; GL, germ-line; NA, not available.

*Gene patterns were determined with J<sub>G</sub>, C<sub>G</sub>, C<sub>R</sub>, or J<sub>H</sub> probes.

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

**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 EcoRI and HindIII and hybridized with the C<sub>G</sub> probe, likewise showed a germ-line pattern (data not shown).

**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 TCR<sub>A</sub> gene complex by looking for mRNA expression of this gene. Mature TCR<sub>A</sub> 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<sub>6</sub> gene be detected (21, 22). In murine thymocytes and cytotoxic T-lymphocyte clones mRNA of ≈1.7 kb containing C<sub>G</sub> sequences has been detected (2, 32).

TCRG and TCRB mRNA was not detected in any of the precursor B-cell lines studied (Table 1). TCR<sub>A</sub> was expressed in LCs 11 and 12, where TCR<sub>G</sub> and TCRB genes were in germ-line form. Mature T-cell leukemia cells (LCs 14–17) all expressed TCRB and TCR<sub>A</sub> while TCRG expression was detected in the patients (Table 2). None of the precursor T-cell lines (LCs 18–21) expressed TCR<sub>A</sub>. 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 TCR<sub>G</sub> had different patterns of TCRB and TCR<sub>A</sub> expression. LC 23 had a germ-line TCRB pattern on Southern analysis and expressed the immature TCRB message (1.0 kb) that lacks V<sub>G</sub> sequences. LC 25 was likewise germ line for TCRB but expressed TCR<sub>A</sub> 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
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, study 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 TCR 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 Jκ-Cα transcripts lacking translation initiation codons can be found in B cells (30). Probes to the Jκ 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 TCRB 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 TCRα 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. TCRα 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 TCRα gene activation. Other investigators have likewise found that activation of TCRB genes precedes TCRα 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. Tα, TCRG; Tα, TCRB; Tα, TCRα.

*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 TCRB gene mRNA, and, for the leukemias studied here, no longer express measurable quantities of TCRG message (except LC14). 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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