REARRANGEMENT AND EXPRESSION OF THE $\alpha$, $\beta$, AND $\gamma$
CHAIN T CELL RECEPTOR GENES IN HUMAN THYMIC
LEUKEMIA CELLS AND FUNCTIONAL T CELLS

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The thymus is believed to play a central role in the development of the T cell. T cell precursors enter the thymus and undergo a differentiation process that results in functional effector cells. Within this organ a number of subsets of thymocytes are recognized; these are believed to represent different stages of the differentiation process (1-3). Individual T cell leukemias and T cell leukemic cell lines possess cell surface markers and intracellular enzyme profiles that are very similar to those of subsets of thymocytes. This has led to the belief that the cells of T cell leukemia cell lines are derived from thymocytes at different stages of differentiation, and due to malignant transformation are arrested at that stage. Based on studies of thymocyte subsets and of T cell leukemias and T cell leukemic cell lines a number of hierarchical differentiation programs have been proposed. However, these classifications do not take into account rearrangement and expression of the T cell antigen receptor genes, which are believed to be ultimately responsible for the antigen specificity of the individual T cell.

The molecular cloning and subsequent studies of the $\beta$ chain (4, 5), the $\alpha$ chain (6-9), and $\gamma$ (10) chain of the T cell antigen receptor have revealed that these genes are distinct but related to the Ig heavy and light chain genes. Like the Ig genes, the genomic organization of the TcR genes consists of noncontiguous, variable, diversity, joining, and constant region segments (11-18). In the $\beta$ chain there are two constant regions ($C\beta 1$ and $C\beta 2$); ~5 kb upstream of each of the constant regions is a cluster of J segments ($J\beta 1$ and $J\beta 2$) (11-13). The $\alpha$ chain gene differs from the $\beta$ chain gene in that there is only one constant region and instead of having only 6-7 J segments clustered together there are more than 20 J segments dispersed over more than 50 kb immediately upstream of the constant region (14-16). The $\gamma$ chain gene in mouse contains three to four constant regions and J segments just upstream of each of the constant regions (17, 18). In man only two constant regions have been recognized (19).

To produce a functional TcR protein the $\alpha$ and $\beta$ chain genes must undergo somatic rearrangement. Although the protein of the $\gamma$ chain gene has not been identified, this gene also undergoes somatic rearrangement. Rearrangement of the $\beta$ and $\gamma$ chain genes can be detected in most cases by the use of probes to
the constant region of these genes (10, 17–24). In some instances the use of genomic probes may help to clarify rearrangement of the β and γ chain genes. Through the use of such probes it has been possible to gather information relating to the structure of the TcR β and γ chain genes in thymic leukemic cells and functional T cell clones. However, the inherent structure of the α chain gene has made such studies more difficult. As rearrangement can occur into any one of the J segments over a region of >50 kb (14–16), the use of probes to the constant region usually do not show rearrangement (8, 9). It should be possible to solve this problem, in part, by using genomic probes to the region of the J segments.

In this paper we have used cDNA and genomic probes to the α, β, and γ chain of the TcR to determine their structure and expression in leukemic T cell lines representing different stages of thymic differentiation. To determine the state of these genes in mature T cells we also examined a number of functional T cell clones. These studies are consistent with a sequential rearrangement and use of first the γ, β, and then the α chain genes. We did not find a direct correlation between the developmental stage of thymic leukemia T cells, as determined by cell surface markers and cellular enzyme levels, and the rearrangement of their TcR α, β, and γ chain genes.

Materials and Methods

Cell Lines. The thymic leukemia cell lines and their characteristics have been previously described (25). The isolation and characterization of functional T cell clones derived from peripheral blood T lymphocytes and expressing either helper or killer activity have also been previously described (26, 27). The functional T cell clones have been found to have allo-reactivity or reactivity to soluble antigens plus MHC gene products.

Southern Blot Analysis. DNA extracted from leukemic cell lines and functional T cells was digested with 5–10 U/μg with one of the restriction enzymes Eco RI, Bam H1 or Hind III. The resulting fragments were separated by electrophoresis through a 0.8% agarose gel, transferred to nitrocellulose, and hybridized with a 32P-labeled probe as described by Southern (28). The probes used were: α chain cDNA (9), constant region of β chain cDNA (4), genomic clones derived from the region of the J segments of the α chain gene (16), a genomic clone of the Jβ2 region of the β chain gene (12), and a γ chain cDNA (a gift from Dr. D. Littman, University of California at San Francisco).

Northern Gel Analysis. Blot hybridization of poly A+ mRNA was performed as previously described (9). The probes used were: α chain cDNA and β chain cDNA.

Results

The Structure and Expression of the T Cell α, β, and γ Chain Genes in T Cell Leukemias of Thymic Origin. Leukemic T cell lines have proven to be useful in determining the stages of T cell differentiation. Three genes that undergo somatic rearrangement and expression predominantly in T cells have been identified. To determine how these events relate to stages of differentiation, as determined by cell surface markers and cellular enzyme levels, we carried out Southern and Northern blot analyses of DNA and RNA from a series of 14 thymic leukemia cell lines; four non–T cell lines were also included. The stage of differentiation of these cell lines is summarized in Table 1.

Structure of TcR β Chain. Previous studies have indicated that there is rearrangement of TcR β chain in almost all T cells. Rearrangement of TcR β may
### TABLE 1

**Leukemia Cell Lines and Their Sources**

| Cell Line   | Type of Cell Line* | Stage of T Cell Differentiation† | Origin       |
|-------------|-------------------|---------------------------------|--------------|
| THP-1       | Myeloid           | —                               | AMOL         |
| HL60        | Myeloid           | —                               | APL          |
| NALM-1      | Pre-B             | —                               | CML          |
| RPMI 3658   | B cell            | I                               | PBL          |
| P30/OKUBO   | T cell            | I                               | ALL          |
| HPB-ALL     | T cell            | II                              | ALL          |
| RPMI 8402   | T cell            | II                              | ALL          |
| DND-41      | T cell            | II                              | ALL          |
| CEM         | T cell            | II                              | ALL          |
| Jurkat      | T cell            | II                              | ALL          |
| MOLT-3      | T cell            | III                             | ALL          |
| MOLT-4      | T cell            | III                             | ALL          |
| P12/ICHI    | T cell            | III                             | ALL          |
| MOLT-16     | T cell            | III                             | ALL          |
| SKW-3       | T cell            | IV                              | CLL          |
| KE-37       | T cell            | IV                              | ALL          |
| MOLT-15     | T cell            | IV                              | AMOL         |
| PEER        | T cell            | IV                              | ALL          |

* Characterization of the cell lines is detailed in reference 25.
† Stages of differentiation are defined in reference 2.

AMOL, acute monocytic leukemia; APL, acute promyelocytic leukemia; CML, chronic myelocytic leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia.

Involve either the first set of J segments or the second set of J segments; the region involved in the rearrangement may be determined using a combination of probes and restriction enzymes.

Rearrangement involving Jβ1 can be determined by hybridizing a constant region probe to Eco RI cleaved DNA on a filter. This results in hybridization to an 11 kb and a 4 kb fragment. Inspection of the restriction enzyme map (Fig. 1) reveals that the 11-kb band contains the Jβ1 and Cβ1 region while the 4-kb band contains the Cβ2 region. Rearrangement of the Cβ1 complex results in the loss of the 11-kb band and the appearance of a new band, which may be either greater than or less than 11 kb in size. Rearrangement into the Cβ2 complex results in deletion of the 11-kb band and the appearance of no new bands; in either case the 4-kb band does not change. The myeloid cell line THP-1 and the thymic leukemia cell line P30/OKUBO were found to have the germline configuration, while new bands were observed in the thymic leukemic cell lines RPMI 8402, DND-41, P12/ICHI, MOLT-16, SKW-3, KE-37, and MOLT-15 and the pre-B cell line NALM-1 (Fig. 2). Therefore, these cells have undergone a rearrangement involving the Cβ1 complex. The T cell lines PEER, MOLT-3, MOLT-4, and HPB-ALL (data not shown) contain only the 4-kb fragment; this suggests that both alleles of Cβ1 have been deleted and rearrangement into the Cβ2 region has occurred.

Rearrangements involving the Cβ2 complex may be detected by probing Hind III cut DNA with a constant region probe. This results in three bands; a 3.3-kb
FIGURE 1. (A) A restriction enzyme map of the human germline genomic α chain gene joining and constant region. The position of the molecular probes used in these experiments is designated. (B) A restriction map of the diversity, joining and constant region of the human β chain T cell receptor gene. The position of the molecular probes used in these experiments are indicated. B, Bam HI; E, Eco RI; H, Hind III and X, Xba I.

FIGURE 2. DNAs extracted from thymic leukemia and control cell lines were digested with the restriction enzyme Eco RI, separated by gel electrophoresis, transferred to nitrocellulose, and hybridized to a constant region probe of the T cell receptor β chain genes.
FIGURE 3. (A) DNAs extracted from thymic leukemia and control cell lines were digested with the restriction enzyme Hind III, separated by gel electrophoresis, transferred to nitrocellulose, and hybridized to a constant region probe of the T cell receptor $\beta$ chain genes. (B) DNAs extracted from thymic leukemia and control cell lines were digested with the restriction enzyme Hind III, separated by gel electrophoresis, transferred to nitrocellulose, and hybridized to a constant region probe of the T cell receptor $\beta$ chain genes.

band that contains C$\beta$1, a 6.0-kb band that contains the 3' end of C$\beta$2, and a 7.5-kb band that contains J$\beta$2 and the 5' end of C$\beta$2 (J$\beta$2-C$\beta$2). Rearrangement into the C$\beta$2 complex results in deletion of the fragment bearing C$\beta$1 and a change in size of the fragment bearing J$\beta$2-C$\beta$2. The cell lines PEER, MOLT-3, MOLT-4, and HPB-ALL have lost both the 3.3-kb and 7.5-kb band and have acquired two new bands (Fig. 3). This indicates that in these cell lines rearrangements involving both alleles of C$\beta$2 have occurred. In the cell lines MOLT-16, RPMI 8402, and DND-41 the germline 7.5- and 3.3-kb bands are present, in addition a new band is present in each case (data not shown). This indicates that only one allele has rearranged to C$\beta$2. The germline configuration is present in the cell lines NALM-1, P30/OKUBO, MOLT-15, and SKW-3, indicating that in these lines rearrangement involving C$\beta$2 has not occurred.

The cell lines P12/ICHI and KE-37 also have a germline configuration,
however, there are three observations that suggest that rearrangement involving Cβ2 has occurred. First, in the Eco RI digest the 11.0-kb band was deleted and only one rearrangement band was seen (Fig. 2). Second, the germline pattern of normal DNA cut with Hind III and probed with a constant region probe gave 7.5- and 3.3-kb bands that are more intense than the 6.0-kb band (data not shown). In the case of P12/ICHI and KE-37 the 6.0-kb band is of the same intensity as the 7.5- and 3.3-kb bands. This may be due to the fact that the rearrangement resulted in a 6.0-kb fragment that overlies the 6.0-kb Cβ2 fragment. Finally in the Bam HI digest two rearranged bands were seen (data not shown). To determine whether a rearrangement involving Cβ2 had occurred we used a genomic probe to the Jβ2 region (see Fig. 1). This probe gives a 4.0-kb and a 7.5-kb band for Eco RI and Hind III cut DNA, respectively. Using either restriction enzyme, rearranged bands were seen in the P12/ICHI, MOLT-16, and KE-37 DNA when probed with the Jβ2 fragment (Fig. 4A). This confirms that rearrangement had occurred into the Cβ2 complex.

Hind III and Eco RI digests of DNA from the rest of the cell lines were also probed with the Jβ2 fragment (representative data in Figs. 4B and 5). THP-1 consistent with the previous studies yielded the expected germline bands, while rearranged bands were detected in RPMI 8402, PEER, MOLT-3, and MOLT-4. HPB-ALL (data not shown) DNA cleaved with Eco RI also showed rearrange-
merit. A germline pattern was observed for both Eco RI and Hind III cleaved DNA from the cell lines NALM-1, SKW-3, and P30/OKUBO.

In DNA from MOLT-3, MOLT-4, and MOLT-16 probed with the Jβ2 fragment extra bands were seen (Figs. 4 and 5). In the cases of MOLT-3 and MOLT-4 cut with either Eco RI or Hind III there were three rearranged bands, while in Hind III cut MOLT-16 DNA there were two rearranged bands and a germline band (data not shown). Eco RI cut DNA from MOLT-16 contained a single rearranged band and a germline band. These results are compatible with there being three rearranged copies of Jβ2, however, results obtained with the Cβ probe are not compatible with this. The most likely explanation is that fragments carrying Jβ2 sequences were excised, and instead of being lost have reintegrated into the genome at another site.

The results of the above studies are summarized in Table II. Rearrangement of TcR β chain was seen in all of the Thymic leukemia cell lines except P30/OKUBO. In addition, rearrangement of TcR β was found in the pre-B cell line NALM-1. In the T cell lines, the number of alleles with rearrangements into the first and second constant regions are 10 and 17 respectively (Table II).

Expression of TcR β in Thymic Leukemia Cell Lines. The expression of TcR β in the thymic leukemia cell lines was examined by Northern blot analysis of poly A+ mRNA hybridized with a β chain constant region probe. β chain mRNA was present in all of the T cell leukemic cell lines except for P30/OKUBO (Table II). Furthermore, in each case, a 1.3-kb full-length transcript was seen. A truncated 1.0-kb transcript containing DJC or JC sequences was detected in some cases.
Table II

Beta Chain Rearrangement and Expression of Leukemic Cell Lines

| Cell line | Beta 1 allele | Beta 2 allele | Rearranged to beta | Beta chain expression |
|-----------|---------------|---------------|--------------------|----------------------|
| THP-1     | g,g           | g,g           | g                  | ND                   |
| HL60      | g,g           | g,g           | g                  | -                    |
| NALM-1    | r,r           | g,g           | 1,1                | ND                   |
| RPMI 3638 | g,g           | g,g           | g                  | -                    |
| P30/OKUBO | g,g           | g,g           | g                  | -                    |
| HPB-ALL   | d,d           | r,r           | 2,2                | +                    |
| RPMI 8402 | r,d           | r,g           | 1,2                | +                    |
| DND-41    | r,d           | r,g           | 1,2                | ND                   |
| CEM       | d,d           | r,r           | 2,2                | +                    |
| Jurkat    | r,r           | g,g           | 1,1                | +                    |
| MOLT-3    | d,d           | r,r           | 2,2                | +                    |
| MOLT-4    | d,d           | r,r           | 2,2                | +                    |
| P12/ICHI  | r,d           | r,r           | 1,2,2              | ND                   |
| MOLT-16   | r,d           | r,g           | 1,2                | +                    |
| SKW-3     | r,d           | g,d           | 1,d                | +                    |
| KE-37     | r,d           | r,r           | 1,2,2              | ND                   |
| MOLT-15   | r,r           | g,g           | 1,1                | +                    |
| PEER      | d,d           | r,r           | 2,2                | ND                   |

Leukemia cell lines are described in Table 1. Rearrangements were evaluated by use of restriction enzymes Eco RI, Hind III, and Bam HI. d, deleted; r, rearranged; 1, rearrangement involving C131; 2, rearrangement involving C132; g, germline configuration.

Structure of TcR α Chain Gene in Leukemic Cell Lines. Similar to the β chain genes, the TcR α chain genes should also undergo somatic rearrangement to assemble a functional gene; demonstration of rearrangement, however, has been hampered by the fact that the region containing the J segments extends over 50 kb. In an attempt to detect rearrangement involving the α chain locus we have used probes derived from the J region. Fig. 1A contains a restriction enzyme map of this region and shows the probes used in the present study.

The first probe (JaA) is a 3.6-kb Bam HI fragment located about 1 kb 5' to the constant region. The results using this probe are shown in Fig. 6. As this fragment contains an alu repeat sequence it was necessary to prehybridize and hybridize the filter with 100 μg/ml of sheared, denatured, unlabeled human DNA. Two leukemic cell lines, Jurkat and HPB-ALL, showed rearrangement with this probe (Fig. 6, A and B). This observation is consistent with the finding that these two cell lines use a J sequence located within this genomic fragment (8, 9).

The next probes, JaB and JaC, are 2.3- and 4.7-kb Eco RI fragments located ~16 kb upstream of the constant region, respectively. Southern blots of DNA digested with Eco RI, Bam HI, and Hind III were examined. No rearrangements were observed in any of the cell lines examined, (data not shown) although both of these fragments were deleted in HPB-ALL (Fig. 7). Deletions were not observed in any of the other cell lines.

The final probe used is JaD, a 2.9-kb Eco RI fragment about 30 kb upstream of the constant region; with this probe, rearrangement and deletion were
observed in a number of cell lines. The cell lines MOLT-16 and SKW-3 showed deletion of the germline band and the presence of a new band, while the cell line HPB-ALL showed deletion of the germline band (data not shown). The cell lines MOLT 3 and MOLT 4 also had deleted the germline band (Fig. 8). A summary of the results using these genomic probes is presented in Table III. Evidence of rearrangement was observed in 6 of the 14 thymic leukemia cell lines. No evidence of rearrangement was seen in the four non-T cell lines.

Expression of TcR α. The above studies indicate that rearrangement of TcR α occurred in 6 of the 14 leukemic cell lines. The inability to detect rearrangement in the eight other cell lines may be due to the fact that we have not yet obtained sufficient probes for detecting rearrangement, or that rearrangement has not occurred. Another manner of determining whether rearrangement has occurred is to examine the mRNA produced. Expression of a 1.6-kb α chain...
RNA containing V, J, and C sequences is dependent upon prior rearrangement of the α chain locus. Northern blots of RNA from the leukemic cell lines was probed with an α chain cDNA (PY1.4); the results of this are summarized in Table III. Expression of a 1.6-kb RNA was found in MOLT-3, MOLT-16, SKW-3, Jurkat, P30/OKUBO, and HPB-ALL. No α chain RNA was detected in RPMI 8402, CEM, MOLT-4, and MOLT-15. The B cell line RPMI 1788 expresses a 1.3-kb truncated message.

Rearrangement and Expression of T Cell γ Chain in Leukemic Cell Lines. The human T cell γ chain consists of two cross hybridizing constant region genes, Cγ1 and Cγ2 (19, 29). Bam HI digestion of non-T cell DNA results in 16- and 3.3-kb bands containing Cγ1 sequences and 11.7- and 4-kb bands containing Cγ2 sequences. A southern blot of Bam HI cleaved DNA from the leukemic cell lines was probed with a γ chain cDNA. The results of these studies are summarized in Table IV. Rearrangement of the γ chain was found in all of the thymic leukemia cell lines except P30/OKUBO. In addition, there was rearrangement of the γ chain gene in the pre-B cell line NALM-1.

The expression of γ chain mRNA was examined by Northern blot analysis of poly A + mRNA hybridized with the γ chain cDNA probe. Consistent with the rearrangement data, γ chain message was found in the cell lines MOLT-3, MOLT-4, Jurkat, MOLT-15, and MOLT-16. No message was found in the cell line P30/OKUBO.

Structure and Expression of the α, β, and γ Chain Genes in Functional T Cell
FIGURE 8. DNA from leukemic cell lines and functional T cell clones was digested with the restriction enzyme Bam HI and examined by Southern blot analysis using JαD region probe as indicated in Fig. 1A.

Clones. As a comparison to the thymic leukemic cell lines the structure and expression of the α, β, and γ chain genes in functional T cell clones were evaluated. 15 functional T cell clones with either helper, killer, or suppressor activity were derived from the same individual. Rearrangement of the β and γ chain genes was found in all of the functional T cell clones examined. We attempted to determine whether there was rearrangement of the α chain region, using the previously described probes. The results of this study are summarized in Table V and representative Southern blots are presented in Fig. 9, A and B. Clear rearranged bands were seen in five of the clones, and deletion of both alleles was observed in five other clones. The fact that all clones were derived from the same individual rules out the possibility that the observed rearrangements were due to an inherited restriction fragment length polymorphism.

To determine whether the functional T cell clones expressed α chain RNA, a Northern blot was probed with an α chain cDNA. RNA from 10 of functional T cell clones was available for this study. In all 10 cases a 1.6-kb α mRNA was detected (Table V). The level of α chain mRNA varied widely, and in several of the clones, in addition to the 1.6-kb transcript, a shorter transcript was also seen.

Discussion

In the present study we have evaluated the structure and expression in thymic leukemia cell lines and functional T cell clones of three genes that undergo somatic rearrangement in T cells. We found that in all but one of the thymic leukemia cell lines, and in all the functional T cell clones examined, rearrange-
### Table III

**Alpha Chain Rearrangement and Expression in Leukemic Cell Lines**

| Cell line | J Alpha probe A | J Alpha probe B | J Alpha probe C | J Alpha probe D | Alpha chain rearrangement conclusion | Alpha chain expression |
|-----------|-----------------|-----------------|-----------------|-----------------|--------------------------------------|------------------------|
| THP-1     | g               | g               | g               | g               | g                                   | ND                     |
| HL60      | --              | --              | --              | --              |                                      |                        |
| NALM-1    | g               | g               | g               | g               | g                                   | ND                     |
| RPMI 8838 | g               | g               | g               | g               | g                                   | ND                     |
| P30/OKUBO | --              | --              | --              | --              |                                      |                        |
| HPB-ALL   | r,r             | d,d             | d,d             | d,d             | r,r                                 | +                      |
| RPMI-8402 | g               | g               | g               | g               | g                                   | ND                     |
| DND-41    | g               | g               | g               | g               | g                                   | +                      |
| CEM       | --              | --              | g               | --              |                                      |                        |
| Jurkat    | g,r             | --              | g               | g               | g,r                                 | +                      |
| MOLT-3    | --              | --              | g               | g               | d,d                                 | +                      |
| MOLT-4    | --              | --              | g               | g               | d,d                                 | +                      |
| P12/ICHI  | g               | g               | g               | g               |                                     |                        |
| MOLT-16   | g               | g               | g               | d,r             | r,r, or r,d                          | +                      |
| SKW-3     | g               | g               | g               | d,r             | r,r, or r,d                          | +                      |
| KE-37     | g               | g               | g               | g               |                                      |                        |
| MOLT-15   | g               | g               | g               | g               |                                      |                        |
| PEER      | g               | g               | g               | g               |                                      | ND                     |

Cell lines are described in Table I. See Table II for abbreviations. Probes are described in Fig. 1A.

Rearrangement of both the $\beta$ and $\gamma$ chain genes had occurred. Rearrangement of the $\beta$ chain gene was detected using a combination of restriction enzymes and cDNA and genomic probes. In this manner it was possible to determine whether rearrangement involved the first or second constant region. Rearrangements involving the first constant region, 10, were less frequent than rearrangements involving the second constant region, 17. The reason for this bias may be due to rearrangement occurring in a sequential order; that is, rearrangement may initially involve $\text{C}_{21}$ or $\text{C}_{22}$. If a rearrangement involving $\text{C}_{21}$ does not produce a functional protein, subsequent rearrangement involving $\text{C}_{22}$ may occur. This bias is also reflected at the RNA level; this was determined by analyzing the frequency of $\text{C}_{21}$ and $\text{C}_{22}$ in a cDNA library prepared from peripheral blood lymphocytes (Kimura, N., The Ontario Cancer Institute, unpublished observation).

In this study and others in which the structure of TcR $\beta$ in T cells has been evaluated, three rearranged bands are occasionally observed. As there are only two alleles, there should only be two bands. During rearrangements, pieces of DNA are usually lost from the genome; the third band may come about as the result of reintegration of the deleted piece of DNA rather than its loss from the cell (30).

The expression of TcR $\beta$ RNA was determined in the leukemic cell lines and in the functional T cell clones. In all cases, a 1.3-kb transcript was found and in some cases a 1.0-kb transcript was also present. The 1.3 kb RNA is the result of full VDJ rearrangement, while the 1.0-kb RNA containing either DJC or JC sequences is the result of either partial rearrangement or aberrant transcription of a nonrearranged gene (9).
TABLE IV
Leukemic Cell Lines Rearrangement and Expression of Gamma, Beta, and Alpha Chain Genes

| Cell line      | γ chain | β chain | α chain* | Stage of differentiation | Stage of differentiation |
|----------------|---------|---------|----------|--------------------------|-------------------------|
| THP-1          | ND      | -       | -        | -                        | -                       |
| HL60           | ND      | -       | -        | -                        | -                       |
| NALM-1         | +       | +       | -        | -                        | -                       |
| RPMI 3638      | -       | -       | -        | -                        | -                       |
| P30/OKUBO      | -       | -       | +        | I                        | I                       |
| HPB-ALL        | +       | +       | -        | II                       | II/III                  |
| RPMI 8402      | +       | +       | -        | II                       | I                       |
| DND-41         | +       | +       | -        | II                       | III                     |
| CEM            | ND      | -       | +        | II                       | I/II                    |
| Jurkat         | ND      | +       | +        | II                       | II                      |
| MOLT 3         | +       | +       | +        | III                      | I/II                    |
| MOLT 4         | +       | +       | +        | III                      | I/II                    |
| P12/ICHI       | +       | +       | -        | III                      | II                      |
| MOLT 16        | +       | +       | +        | III                      | -                       |
| SKW-3          | +       | +       | +        | IV                       | I/II                    |
| KE-37          | +       | +       | -        | IV                       | III                     |
| MOLT 15        | +       | +       | -        | IV                       | -                       |
| PEER           | ND      | +       | -        | IV                       | II                      |

* α chain was scored as + if either the gene showed evidence of rearrangement or mRNA was detected.
* See reference 2.
* See reference 1.

TABLE V
Alpha Chain Rearrangement and Expression in Functional Cell Lines

| Cell Line* | J Alpha probe A | J Alpha probe B | J Alpha probe C | J Alpha probe D | Alpha chain rearrangement conclusion | Alpha chain expression |
|------------|-----------------|-----------------|-----------------|-----------------|--------------------------------------|------------------------|
| Autologous LBCL§ | g               | -               | g               | g               | r,r, or r,d                          | ND                     |
| FG         | g               | g               | d,d             | r,r, or r,d     | r,r, or r,d                          | ND                     |
| Clone 14   | g               | g               | d,d             | r,r, or r,d     | r,r, or r,d                          | +                      |
| Clone 19   | g               | g               | g               | g               | g                                     | ND                     |
| Clone 22   | g               | g               | g               | r,g             | r,g                                  | ND                     |
| Clone 46   | g               | g               | g               | r,g             | ND                     |
| Clone 78   | g               | g               | g               | r,g             | ND                     |
| Clone 80   | g               | g               | d,d             | r,r, or r,d     | +                      |
| Clone 87   | g               | g               | d,d             | r,r, or r,d     | +                      |
| Clone 89   | g               | g               | d,d             | r,r, or r,d     | +                      |
| Clone 179  | g               | g               | g               | r,g             | ND                     |
| Clone 197  | g               | g               | d,d             | r,r, or r,d     | +                      |
| Clone 207  | g               | g               | g               | r,g             | ND                     |
| Clone 209  | g               | g               | g               | r,g             | +                      |
| Clone 243  | g               | g               | d,d             | r,r, or r,d     | +                      |
| Clone 320  | g               | g               | d,d             | r,r, or r,d     | +                      |

* Functional cell lines are described in Reference 26 and 27.
* Probes used are described in Figure 1A.
* Autologous LBCL, is a lymphoblastoid cell line derived from the same individual from whom the functional clones were developed.
Rearrangement of the \( \alpha \) chain locus has been difficult to show. In the present study, we have shown the utility of using genomic probes isolated from the J bearing region of the gene to detect or infer rearrangement. In both the leukemic T cells and functional T cell clones it was possible to find new rearranged bands. The fact that the functional T cells were derived from the same individual provides evidence that we were detecting rearrangement and not an inherited restriction fragment length polymorphism. It is also possible to infer that rearrangement has occurred by detecting gene deletion. Gene deletion is recognized either by the total loss of hybridization to a germline fragment or the reduction in intensity of the germline fragment; the latter requires appropriate internal controls. In some of the cases studied the most 5' region was deleted, while the probe just 3' to this revealed hybridization. In such cases it is likely that the rearrangement had occurred to a J segment between these two probes. This can be confirmed by the use of more probes or by the use of restriction enzymes that cut the DNA infrequently and the subsequent separation of the DNA fragments on an appropriate agarose gel. In the small sample studied here it appears that the whole region of Js may be involved in rearrangements.

The expression of \( \alpha \) chain mRNA was determined by probing Northern blots with an \( \alpha \) cDNA. Messenger RNA corresponding to the \( \alpha \) chain was detected in...
only 6 of the 10 thymic leukemia cell lines tested but in 10 of 10 functional T cell clones tested. In the functional T cell clones, α gene rearrangement was detected in eight of nine cases in which both α chain rearrangement and expression was determined. If one assumes that all of the functional T cell clones express α mRNA then it was possible to detect rearrangement in 10 of the 13 instances in which rearrangement occurred. Thus the available probes to the J region are capable of detecting >75% of the expected rearrangements. In the thymic leukemia cell lines α mRNA was detected in six cell lines; gene rearrangement was detected in five of these lines. Based on the above results, the cases in which there was no gene expression and no rearrangement of the α chain locus likely have not rearranged their α chain gene. Thus, in the leukemic cell lines we have identified three different groups: one that has not rearranged γ, β, or α chain genes (P30/OKUBO), another group that has rearranged γ and β chain genes, but not α chain genes (RPMI 8402, DND-41, CEM, P12/ICHI, KE-37, MOLT-15, and PEER), and a third group that has rearranged all three genes (HPB-ALL, Jurkat, MOLT-3, MOLT-4, MOLT-16, and SKW-3) (Table IV). This last pattern was also found in mature functional T cells. Comparing these three subgroups, based on molecular findings, to the subgroups defined by cell surface markers and cellular enzymes a number of discrepancies are evident (1–3). These may be due in part to the fact that the cells being studied are leukemic cell lines and that the expression of cell surface antigens may be aberrant (31).

The cell line P30/OKUBO, which has not rearranged α, β, or γ chain genes, is of particular interest as it may represent the earliest stage of T cell intrathymic development. The only T cell marker that it expresses is the cell surface antigen T1. Recently it has been reported that the Ig heavy chain genes of this cell are rearranged (23). This latter finding raises questions as to the lineage of P30/OKUBO, as it may represent the earliest stages of T cell or B cell development. Further studies of this cell line are necessary to resolve this issue.

As the cell lines MOLT-3 and MOLT-4 were derived from the same individual at two different times comparison of the results obtained in the present study may provide some insight into the stability of TcR gene rearrangement and expression: Identical gene rearrangements for α, β, and γ chain genes are present in both cell lines. As well, both cell lines express TcR β chain and γ chain mRNA. However, while MOLT-3 expresses α chain mRNA, MOLT-4 does not. These two cell lines have been carried in culture for several years and it is not known whether the difference existed in the patient or developed during passage of the cells. Regardless, this comparison indicates a relative stability of TcR gene rearrangement and the variable nature of gene expression in leukemic cells.

Finally, in addition to defining the possible order of rearrangement in leukemic T cells, we have described a series of probes derived from the J region of the TcR α chain gene that are useful in detecting rearrangement of the α chain gene and may be of value in detecting chromosome translocations involving the α chain locus. In association with probes to the β and γ chain genes it has been possible to identify three stages of T cell development.

Summary

Using cDNA and genomic probes representing the α, β, and γ chain of the human T cell receptor genes, we have examined the structure and expression of
these genes in 14 human leukemic T cell lines, representing different stages of thymic differentiation, and 15 functional human T cell clones. Rearrangement of the γ and β chain genes was found in all of the functional T cell clones and all but one (P30/OKUBO) thymic leukemia cell line; all of the lines that had rearrangement of the β chain expressed β mRNA. Expression of the α chain was found in all of the functional T cell clones examined, while rearrangement of the α chain gene, using currently available probes to the J region, could be shown in 10 of 13 functional clones. In contrast, expression of the α chain was found in 6 of 10 leukemic T cell lines, while rearrangement was found in six of these nine cell lines. Of the 14 leukemic cell lines studied for rearrangement of the α chain, rearrangement was found in six cases. The data obtained with the cell lines are consistent with an ordered rearrangement and expression of the γ, β, and α chains of the T cell antigen receptor (TcR) genes.

The leukemic cell lines used in the present study have previously been characterized with regard to cell surface antigens and intracellular enzymes. Based on those results a scheme of thymic development was proposed. The developmental stages identified by those studies are not in complete agreement with stages of T cell development, as determined in the present study using molecular probes.

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