Different T-Cell Receptor Gene Configurations in T-Cell Neoplasms and Acute Lymphoblastic Leukemia

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ABSTRACT

Southern blotting and multiple restriction enzymes were used to analyze T-cell receptor (TCR) and immunoglobulin heavy chain genes in 20 postthymic T-cell neoplasms, ten prethymic and thymic T-cell tumors, and 45 cases of precursor-B acute lymphoblastic leukemia (ALL). Immunoglobulin heavy chain, never rearranged in a postthymic specimen and only once in a prethymic/thymic sample, was rearranged in all but two cases of precursor-B ALL. In contrast, biallelic rearrangement of TCRγ with deletion of the first constant region germline fragment was regularly seen in T-cell neoplasms, but only twice in the 45 precursor-B ALL cases. TCRα, was rearranged in all but one postthymic sample, in all prethymic/thymic samples, and in approximately half of precursor-B ALL specimens as well. Preferential use of Vγ regions was evident among the various disorders: V8 and V10 in postthymic neoplasms; and V3, V5, V7, and, particularly, V9 in precursor-B ALL. In all the studied conditions, TCRγ was rarely in germline configuration. Extensive biallelic deletion of Jα1, Jα2, and Cγ almost always (19 of 20 specimens) present in postthymic neoplasms, was observed in only a minority (14 of 45) of precursor-B ALL samples. In precursor-B ALL, rearranged antigen receptor genes were more frequently found in common acute lymphoblastic leukemia antigen-positive and terminal deoxynucleotidyl transferase-positive specimens. Furthermore, TCR gene rearrangement in that disorder was characterized by a hierarchical pattern: TCRα was not rearranged without TCRβ or TCRγ. Despite uncertainty of the mechanism, the various disorders can be distinguished on the basis of characteristic antigen receptor gene patterns.

INTRODUCTION

Several earlier investigations suggest that lineage-inappropriate rearrangement of antigen receptor genes occurs frequently in neoplasms of B- and T-lymphocytes (1–3). The present study was undertaken to extend the growing body of experimental evidence that indicates a need to revise that view. In prior studies, we (4) and others (5) rigorously examined TCRγ gene status in neoplasms of mature B-cells. The only inappropriate rearrangements detected were rare, incomplete DJ rearrangements of the TCRγ gene. In this paper we report that immunoglobulin gene rearrangements are absent from 20 neoplasms of postthymic T-cells, a group of tumors more difficult to assemble. Precursor-B ALL is the disorder in which concomitant (inappropriate) immunoglobulin and TCR gene rearrangement has been most frequently reported. Rearrangement of the immunoglobulin heavy chain gene is accompanied by rearrangement of the TCRγ gene in 80 to 90% of cases, the TCRα gene in 40 to 50%, and the TCRβ gene in 20 to 30% (6–10). However, reports of earlier investigators suggest that the TCR gene alterations in precursor-B and T-ALL can be distinguished by differing preferential use of certain gene segments in the two conditions (10–16). In the work described here, differences in TCR gene status between the two disorders are expanded, and the findings in both are compared with those of postthymic T-cell neoplasms. We find that the biallelic rearrangement of TCRγ characteristic of T-cell neoplasia is rarely seen in precursor-B ALL. We also report differing preferential use of Vγ gene segments in the several disorders. Finally, we observe that the extensive biallelic deletion of TCRγ seen in almost all postthymic T-cell neoplasms is present in only a minority of precursor-B ALL specimens. Thus, the work reported here and that of earlier investigators indicates that precursor-B ALL, postthymic T-cell neoplasia, and prethymic and thymic T-cell neoplasia can be distinguished by comprehensive study of antigen receptor genes. Indeed, it is plausible that such genetic analysis furnishes the most precise criteria for diagnosis and subclassification of ALL; i.e., inappropriate gene findings may signal disease nonhomogeneity or misdiagnosis.

MATERIALS AND METHODS

Patients and Surface Marker Analysis. Thirty patients with T-cell neoplasms were investigated. Diagnosis was based on accepted clinical and phenotypic criteria. This group comprised 20 patients with postthymic T-cell neoplasms (6 with adult T-cell leukemia/lymphoma, 5 with cutaneous T-cell lymphoma, 3 with T-cell chronic lymphocytic leukemia, and 6 with T8-lymphocytosis (large granular lymphocytic leukemia) and 10 with thymic or prethymic proliferations (7 with T-cell acute lymphoblastic leukemia and 3 with lymphoblastic lymphoma). Studies of TCRα and TCRγ, in some of these patients were reported as part of an earlier investigation (4).

The diagnosis of precursor-B ALL was made in 45 patients on the basis of the cell morphology of a Wright-stained smear, the myeloperoxidase reaction, and cell surface marker analysis. The presence of 5% or more myeloperoxidase-positive cells or more than 30% of cells reactive with one or more anti-myeloid monoclonal antibodies (OKM1, MY4, MY7, or MY9) excluded that diagnosis.

Surface marker analysis was performed on viable mononuclear cells obtained from blood or bone marrow aspirate (ALL) of consecutive untreated patients by centrifugation through a Ficoll-Hypaque gradient. The following surface markers were analyzed (monoclonal antibodies obtained from Ortho Pharmaceuticals, Raritan, NJ, and Coulter Diagnostics, Hialeah, FL): CD2 (OKT11); CD3 (OKT3); CD4 (OKT4); CD5 (OKT1); CD8 (OKT8); CD10 (CALLA); CD11 (OKM1); CD13 (MY7); CD14 (MY4); CD15 (MY9); CD19 (B4); and CD20 (B1); and HLA-DR. The indirect fluorescent antibody technique utilizes a fluorescein-conjugated F(ab')₂ goat fraction of anti-mouse γ-globulin antibody and a fluorescence microscope. The enzyme TdT was determined by an indirect immunofluorescence assay using reagents obtained from the Bethesda Research Laboratory (Rockville, MD). Surface immunoglobulin was assessed with fluorescein-conjugated heteroantisera specific for the human IgM and IgG heavy chains and the κ and light chains (Melo Laboratories, Springfield, VA). The methods have been described in detail (17).

Genomic Blot Hybridizations. Six to 10 µg of high-molecular-weight DNA, prepared from each patient's purified bone marrow mononuclear
cells, were digested with three or more appropriate restriction enzymes (\textit{BamHI}, \textit{EcoRI}, \textit{SalI}, \textit{HindIII}, and \textit{BglII} obtained from New England Biolabs, Beverly, MA) and subjected to electrophoresis on 0.8% agarose gel slabs. After denaturation and neutralization, the DNA was transferred to nitrocellulose paper by the technique of Southern (18). Hybridization was carried out at 68°C in a solution of 5x SET, 1x Denhardt's solution, 0.5% SDS, 10% dextran sulfate, 20 mM NaH2PO4, (pH 7.0), 40 μg/ml of salmon sperm DNA, and 0.025 to 0.10 μg of oligonucleotide (Pharmacia, Piscataway, NJ). The DNA probe was labeled with \textsuperscript{32}P (10' dpm). The following human T-cell receptor probes were used: a \textit{BglII/EcoRV} complementary DNA fragment of YT35 specific for the constant region of \textit{TCR}\beta (19); \textit{Jg}, a complementary DNA probe specific for the joining region of \textit{TCR} (20); and \textit{J}, S16, R21XH, and R21EE, specific, respectively, for the first joining region, the second joining region, and the constant region of \textit{TCR} (21). Probes for the immunoglobulin heavy-chain joining (\textit{JH}) region (22) and the \textit{\gamma} chain gene and the genes for the \textit{\beta} and \textit{\gamma} chains of the T-cell receptor in 20 postthymic neoplasms, 10 prethymic and thymic neoplasms, and 45 patients with precursor-B ALL. Findings in 100 previously studied cases of B-cell lymphoma and B-cell chronic lymphocytic leukemia (4) are included.

\textbf{Immunoglobulin Heavy Chain Configuration.} The immunoglobulin heavy chain gene was in germline configuration in the 100 mature B-cell cases (4) studied; in the remainder, one (9 of 45 cases) or both (12 of 45 cases) alleles were rearranged. In contrast, precursor-B ALL closely resembled the mature B-cell proliferations in the regular rearrangement of immunoglobulin heavy chain. Thus, the immunoglobulin heavy chain gene was rearranged in 43 of the 45 precursor-B ALL cases studied; in 38 cases, 2 rearranged alleles were detected.

\textbf{TCR\alpha Configuration.} TCR\alpha gene findings were almost reciprocal to the immunoglobulin heavy chain results. TCR\alpha was rearranged in the 20 postthymic tumors studied; both alleles in 19. That gene was also rearranged in all 10 prethymic and thymic proliferations; both alleles in 6. The configuration of TCR\beta was germline in 38 of the 45 precursor-B ALL samples, a single allele was rearranged in 5, and both alleles in 2. In addition, the first constant region of TCR\beta (an 11-kilobase fragment readily identified in \textit{EcoRI} digests) was deleted in 19 of 20 postthymic samples, 9 of 10 prethymic or thymic samples, but in only the 2 cases of precursor-B ALL with biallelic rearrangement (Fig. 1). The precursor-B ALL findings diverge only slightly from 100 previously studied (4) mature B-cell proliferations in which only 2 partial (DJ) rearrangements were detected.

\textbf{TCR\beta Configuration.} Biallelic rearrangement of TCR\beta was identified in 15 of the 20 postthymic tumors studied, monoallelic rearrangement in 4, and a germline configuration in one. Similarly, both alleles of TCR\beta were rearranged in 9 of the 10 prethymic and thymic proliferations examined, and a single allele in the tenth. Varying configurations of TCR\beta were observed in precursor-B ALL. In approximately one-half of studied cases (24 of 45 cases), a germline condition was demonstrated; in the remainder, one (9 of 45 cases) or both (12 of 45 cases) alleles were rearranged. This T-cell receptor gene was always in germline configuration in the 100 mature B-cell proliferations previously studied (4).

While TCR\beta is rearranged in postthymic, prethymic, and thymic specimens and in approximately half of the precursor-B ALL samples, the pattern of \textit{V} region usage differs significantly in the various disorders. Specific \textit{V} usage is most easily identified in \textit{EcoRI} digests (Fig. 2). These findings are summarized in Fig. 3. The \textit{V}, designations for Forster and coworkers (12, 25, 26) are used.

Postthymic specimens are conspicuous in the utilization of

\begin{table}[h]
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\begin{tabular}{|l|l|l|l|l|l|}
\hline
\textbf{Disorder} & \textbf{Gene} & \multicolumn{2}{c|}{\textbf{Germline}} & \textbf{One allele rearranged} & \textbf{Both alleles rearranged} \\
\hline
\textbf{T-cell*} & & \textbf{No.} & \textbf{No.} & \textbf{No.} & \textbf{No.} \\
\hline
\textbf{Postthymic} & Immunoglobulin heavy chain & 20 & 0 & 1 & 19 \\
\textbf{Precursor-B ALL} & Immunoglobulin heavy chain & 45 & 2 & 4 & 35 \\
\textbf{B-cell lymphoma, B-CLL} & Immunoglobulin heavy chain & 95 & 3 & 3 & 59 \\
\hline
\textbf{T-cell} & & & & & \\
\hline
\textbf{Postthymic} & \textit{TCR}\alpha & 20 & 0 & 1 & 19 \\
\textbf{Precursor-B ALL} & \textit{TCR}\alpha & 10 & 0 & 4 & 6 \\
\textbf{B-cell lymphoma, B-CLL} & \textit{TCR}\alpha & 45 & 38 & 5 & 2 \\
\hline
\textbf{Postthymic} & \textit{TCR}\beta & 20 & 1 & 5 & 15 \\
\textbf{Precursor-B ALL} & \textit{TCR}\beta & 20 & 0 & 4 & 9 \\
\textbf{B-cell lymphoma, B-CLL} & \textit{TCR}\beta & 45 & 24 & 53 & 12 \\
\hline
\textsuperscript{*}Note: \textit{thymic} and \textit{prethymic} include T-cell acute lymphoblastic leukemia and lymphoblastic lymphoma; \textit{postthymic} includes cutaneous T-cell lymphoma, Adult, T-cell leukemia/lymphoma, T8 lymphocytosis, and T-cell chronic lymphocytic leukemia, B-CLL, B-cell chronic lymphocytic leukemia.
\end{tabular}
\caption{\textbf{Antigen receptor genes in leukemia and lymphoma}}
\end{table}
Fig. 1. Configuration of the first constant region gene segment (C1) of TCRs in postthymic T-cell neoplasms (A: Lanes 1 to 6, adult T-cell leukemia/lymphoma; Lanes 7 to 11, cutaneous T-cell lymphoma; Lanes 12 to 14, T-CLL; Lanes 15 to 20, T8 lymphocytosis), prethymic and thymic T-cell neoplasms (B: Lanes 1 to 6, T-ALL; Lanes 7 and 8, lymphoblastic lymphoma), and precursor-B ALL (C: Lanes 1 to 17). Following digestion with EcoRI, the samples were hybridized with a probe for the TCRv constant region gene segment. Rearrangements of C1 are indicated by solid arrows, an 8-kilobase product of incomplete digestion of C2 by open arrows, and the germ-line positions of C1 (11 kilobases) and C2 (4 kilobases) by solid bars. The regular deletion and rearrangement of C1 in T-cell neoplasms (A and B) contrast with the germline configuration in all but one of the precursor-B ALL specimens (C). kb, kilobase.

Fig. 2. Preferential use of TCRv, variable (V) region gene segments in postthymic T-cell neoplasms (A: Lanes 1 to 6, adult T-cell leukemia/lymphoma; Lanes 7 to 11, cutaneous T-cell lymphoma; Lanes 12 to 14, T-CLL; Lanes 15 to 20, T8 lymphocytosis), prethymic and thymic T-cell neoplasms (B: Lanes 1 to 6, T-ALL; Lanes 7 and 8, lymphoblastic lymphoma), and precursor-B ALL (C: Lanes 1 to 17, precursor-B ALL; Lane 18, polyclonal pattern in thymus specimen). After removing the TCRv probe, the same EcoRI-digested samples depicted in Fig. 1 were rehybridized with a probe for the joining region of TCRv. The solid bars identify the germline position of the gene segments; the solid arrowheads, the various Vr fragments; and the small arrows in C, rearranged fragments clearly visible on the original. V4, which can be distinguished from V2 after digestion with BamHI, was present in only a single sample. The preferential use of V8 and V10 in postthymic T-cell neoplasms (A) should be compared with the preferential use of V3, V5, and V9 in precursor-B ALL (C). kb, kilobase.
V8 and V10 and the nonutilization of V3, V5, and V7. Precursor-B ALL utilizes V9 conspicuously, as well as V3, V5, and V7. Thymic and prethymic neoplasms utilize Vα gene segments without apparent discrimination. V2 and unidentified Vα segments (rearrangements signified by X in Fig. 3, appreciated in *BamHI* digests but in neither *EcoRI* or *HindIII* digests) are without apparent discrimination. V2 and unidentified Vα segments (rearrangements signified by X in Fig. 3, appreciated in *BamHI* digests but in neither *EcoRI* or *HindIII* digests) are without apparent discrimination.

**Fig. 3.** Histogram of Vα usage in postthymic T-cell neoplasms (top), prethymic and thymic T-cell neoplasms (middle), and precursor-B ALL (bottom). X fragments are unidentified. This figure is based on all studied samples and includes specimens not illustrated in Fig. 2.

Extensive biallelic deletion was observed in only 14 of 35 (31%) precursor-B ALL specimens (Table 2; Fig. 4B). The majority (58%) of these acute leukemias exhibit rearrangement of one allele (51%), with (13%) or without (38%) deletion of the second allele, or rearrangement of both alleles (7%). Only 5 of the 45 precursor-B ALL specimens exhibited a germline configuration of TCRα. The small number of thymic and prethymic cases revealed a TCRα, picture intermediate between those of postthymic and precursor-B ALL proliferations: 5 of 10 revealed extensive biallelic deletion; 3 a single rearranged allele; and 2 biallelic rearrangement.

It is noteworthy that a single rearrangement accounted for 17 of the 29 (59%) rearranged alleles detected in precursor-B ALL (Table 3; arrow in Fig. 4B). This rearranged fragment also accounted for one of the 7 rearranged alleles in the thymic and prethymic group. The size of this fragment following *BglII*, *BamHI*, and *HindIII* digestion identified it as the same incomplete Vα2-(D)-D3 joining described by others (10, 14, 16, 27). However, pairing of this Vα2 joining with a Vα9 gene segment, as previously reported to occur in normal peripheral γδ T-cells (28–30), was observed in only 4 of our 45 precursor-B ALL specimens. In 13 samples, Vα2 was present without Vα9; in 8 samples, Vα9 without Vα2; and in 20 samples, neither was present.

**Interrelationships among Common Acute Leukemia Antigen, Terminal Deoxynucleotidyl Transferase, and Antigen Receptor Genes in Precursor-B ALL.** Table 4 summarizes the relationships among CALLA, TdT, and the various antigen receptor genes in precursor-B ALL. All 4 of the antigen receptor genes studied were more consistently rearranged in CALLA-positive specimens than in those lacking the antigen. Thus, immunoglobulin heavy chain and TCRα were rearranged in all CALLA-positive specimens, TCRγ in more than half, and TCRδ in nearly one-fourth. In contrast, immunoglobulin heavy chain was rearranged in only 5 of the 7 CALLA-negative samples, TCRγ in only 2, TCR, in only one, and TCRδ in none. There was similar but less marked association of germline antigen receptor genes with negative TdT status. The 7 CALLA-negative samples overlapped only two of the 4 TdT-negative specimens.

A hierarchical order was readily appreciated among the various antigen receptor gene rearrangements. None of the T-cell receptor genes were rearranged in the two leukemia samples with germline immunoglobulin heavy chain configuration. Among the TCR genes, TCRγ was not rearranged without TCRα, nor TCR, without TCRγ.

**DISCUSSION**

With the initial descriptions of immunoglobulin gene rearrangements in B-lymphocytes (31–33) and T-cell receptor gene rearrangements in T-cells (19, 34), it was hoped that antigen receptor genes could provide inviolate markers of lymphocyte lineage and clonality. Unfortunately, the initial findings were followed by numerous reports of lineage-inappropriate

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**Table 2** T-cell receptor α chain genes in leukemia and lymphoma

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* R, rearranged alleles; G, germline allele; D, deleted allele; DD, extensive deletion of both J1, J2, and Cα alleles; B-CLL, B-cell chronic lymphocytic leukemia.

**Table 3** Rearranged T-cell receptor α chain alleles

<table>
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<tr>
<th>Disorder</th>
<th>Total no. of rearranged alleles</th>
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<tr>
<td>Precursor-B ALL</td>
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<td>17</td>
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* V, D, and J indicate, respectively, the variable, diversity, and joining gene segments.
Fig. 4. Configuration of the TCRγ gene in postthymic T-cell neoplasms (A) and precursor-B ALL (B). As a reference standard for the strength of the germline signal, samples from patients with B-CLL were placed in the even-numbered lanes of A and in Lanes 3, 8, 12, 17, 22, and 28 of B. The odd-numbered lanes of A are occupied by DNA specimens from patients with postthymic T-cell neoplasms, and the remaining lanes in B with precursor-B ALL samples. Specimens were digested with BglII and hybridized with a probe for Jγ. Contaminating normal cells are responsible for the residual radioactive signal obtained with DNA from T-cell samples. The same deletional pattern is observed following hybridization with probes for Jγ2 and Cγ. After BglII digestion, the predominant Vγ2-(D)-Dγ3 rearrangement in precursor-B ALL is a 9.9-kilobase fragment (arrow). Solid bars indicate germline position of gene segments.

antigen receptor gene rearrangements. Though cross-lineage gene rearrangements were observed most frequently in precursor-B ALL (6–10), inappropriate antigen receptor gene rearrangements in acute nonlymphocytic leukemia (35–39) and in neoplasms of mature B- and T-cells (40–42) were occasionally reported.

Recently, this puzzling phenomenon of indiscriminate lineage-inappropriate antigen receptor gene rearrangements has been increasingly bounded and qualified by more detailed study. Thus, only rare, incomplete (DJ) rearrangements of TCRγ are detectable in mature B-cell neoplasms, and rearrangements of TCR, have not been identified in these tumors (4, 5). Because neoplasms of mature T-cells are much less frequently encountered, their antigen receptor gene status is less precisely defined. Nonetheless, in the present investigation we detected no rearrangements of immunoglobulin heavy chain in 20 postthymic specimens, even after exhaustive study with 4 appropriate restriction enzymes (Table 1).

Precursor-B ALL is the disorder in which concomitant rearrangement of immunoglobulin and TCR genes is most commonly reported (6–10). However, with careful investigation, the TCR gene status of precursor-B ALL is usually distinguishable from neoplasms of demonstrable T-lineage. Thus, while TCRγ rearrangements are observed in 20% to 30% of cases of precursor-B ALL (6–10), the biallelic rearrangement with deletion of the EcoRI fragment bearing the first constant region, which is a regular finding in T-cell neoplasms, is less frequently observed (Ref. 7; Fig. 1). In this analysis, biallelic rearrangement of TCRγ with deletion of C1 was seen in only 2 of 45 cases of precursor-B ALL, while either double rearrangement or C1 deletion, usually both, was detected in each of the 30 prethymic, thymic, and postthymic neoplasms we studied. Indeed, the infrequency with which we observed biallelic TCRγ rearrangement with C1 deletion in precursor-B ALL raised the possibility that such cases represent unidentified early T-lineage. Unfortunately, the two biallelic cases were studied before we incorporated CD7 into our diagnostic panel. It should be noted that other investigators report a higher

Table 4 Antigen receptor genes in precursor-B acute lymphoblastic leukemia

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* G, germline condition of both alleles; R, rearrangement of one or both alleles; D, extensive deletion of both alleles.

See Table 1 for other details.
incidence of such biallelic rearrangements (6–10), a discrepancy which we cannot resolve.

TCRγ is rearranged in 30% to 50% of precursor-B ALL specimens (6–12). Several groups of investigators report features that distinguish the TCRγ rearrangements of precursor-B ALL from those of T-ALL. Thus, C2 and J2 are preferentially used in T-ALL, while C1 and J1 are preferred in precursor-B ALL (11–14). Furthermore, TCRγ rearrangements tend to be biallelic in T-ALL, and monoallelic in precursor-B ALL. We find that postthymic neoplasms prefer V8 and V10 and that precursor-B ALL prefers V3, V5, V7, and particularly V9; thymic and prethymic tumors use all V regions, and all three categories utilize V2. Our investigation is consistent with the earlier findings in ALL (43–46), but extends the observations to a large group of postthymic neoplasms.

TCRγ is regularly rearranged in precursor-B ALL (80% to 90% of cases) (8–10). Indeed, TCRγ is almost as frequently rearranged in this condition as is the immunoglobulin heavy chain gene. In the present investigation, TCRγ remained in germline configuration in only 5 of the 45 precursor-B ALL cases studied and immunoglobulin heavy chain in 2. Other workers have reported features that distinguish TCRγ rearrangement in this acute leukemia from the alterations of the gene in T-ALL. Thus, V1 and J1 are preferentially used in T-ALL, while V2 is frequently used in precursor-B ALL (10, 16). Indeed, more than half of the precursor-B ALL TCRγ rearrangements involve a single incomplete V2-(D)-D3 joining (10), and the remainder either an incomplete D1/2-D3 joining or other incomplete joinings; complete V2-(D)-J1 fragments have not been detected (10). The present analysis of 20 postthymic neoplasms extends these findings. We find striking, extensive biallelic deletion of J1, J2, and Cγ in 19 cases. In contrast to postthymic cases, this extensive biallelic deletion was detected in only 30% of our precursor-B ALL specimens and in 50% of thymic and prethymic T-cell samples.

Thus, a variety of characteristics distinguish the T-cell rearrangements of precursor-B ALL from those of T-cell neoplasia. Biallelic rearrangement of TCRγ, with deletion of C1, regularly present in T-cell cases, is rarely found in precursor-B ALL. Different V, C, D, and J fragments of TCRγ, and TCRδ are preferentially used in the several conditions. Extensive biallelic deletion of TCRγ is uniform in postthymic neoplasia, while incomplete V2 joinings are characteristic of precursor-B ALL.

In this investigation, the hierarchical pattern of CALLA positivity and TCR gene rearrangement described by other investigators (6–10) was confirmed. We did not encounter cases of rearranged TCRγ in the absence of rearranged TCRα, nor cases of rearranged TCRγ in the absence of rearranged TCRδ. We also agree that antigen receptor genes are more likely to be rearranged in CALLA-positive than CALLA-negative precursor-B ALL (6). Nonetheless, without a more precise picture of the ontogeny of normal α/β and γ/δ T-cells and their interrelations, it is premature to ascribe this hierarchy to a recapitulation of normal development. In this connection, our failure to observe in precursor-B ALL the pairing of Vγ9 and Vδ2 gene segments seen in 60% of normal peripheral γ/δ T-cells (28–30) should be noted. T-cell receptor gene rearrangement in precursor-B ALL could reflect recombinase error (47, 48) or gene rearrangement in an uncommitted lymphoid cell. At the present time, it is possible to ascribe the disparate TCR findings in T-cell neoplasia and precursor-B ALL to legitimate characteristics of their respective lymphocyte lineage and maturity, or to aberrant genetic alterations secondary to their neoplastic state.

Despite the uncertainty about the mechanism, the various disorders can be distinguished on the basis of characteristic patterns of immunoglobulin heavy chain, TCRα, TCRγ, and TCRδ genes. Further, exceptional cases of precursor-B ALL are so few that it is as plausible to ascribe them to faulty clinical diagnosis or disease heterogeneity as to inappropriate antigen receptor gene findings. Indeed, it is reasonable to question the diagnosis of precursor-B ALL in CALLA-negative proliferations, in which all antigen receptor genes are in germline configuration, or in CALLA-positive specimens, with biallelic rearrangement of TCRγ and TCRδ, and deletion of TCRδ. With the spectrum of antigen receptor gene findings in lymphoid neoplasia firmly established, the clinical significance of these genetic findings should be evaluated prospectively in precursor-B ALL by large-scale clinical trial.

REFERENCES

19. Minden, M. D., Toyonaga, B., Ha, K., Chin, B., Yanagi, Y., Gelfand, E.,


Different T-Cell Receptor Gene Configurations in T-Cell Neoplasms and Acute Lymphoblastic Leukemia

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