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Somatic rearrangement of T-cell antigen receptor gene in human T-cell malignancies

(human T-cell receptor/T-cell leukemia)

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ABSTRACT **A cDNA clone representing the gene encoding the β chain of the human T-cell antigen receptor has been isolated recently. By using fragments of this cDNA as hybridization probes in Southern blot analysis of restriction endonuclease-digested genomic DNA, we have now examined the structure of the gene in DNA from 26 patients with acute leukemia and from 23 normal individuals. We have found that the T-cell antigen receptor gene has undergone somatic rearrangement in 14 of 14 patients with the phenotypic diagnosis of T-cell acute lymphoblastic leukemia. In this group of patients, similar patterns of rearrangement appear to occur in different patients. This finding suggests that there is either a limited repertoire of possible rearrangements or an association between the development of leukemia and specific patterns of rearrangement. DNA from 6 patients with acute myeloblastic leukemia, 6 patients with non-B, non-T acute lymphoblastic leukemia, and 23 nonleukemic individuals showed no rearrangement or polymorphism. One case of T-cell acute lymphoblastic leukemia, however, showed rearrangement of both the T-cell receptor β chain and the constant region of the immunoglobulin gene. Studies with mixtures of DNAs from leukemic bone marrow cells and cultured skin fibroblasts, as well as with remission and relapse marrow DNAs from the same patients, indicate that this technique can detect 1% leukemic cells in a mixed population. In addition, DNA from the marrow of a patient in relapse contains a similar rearrangement to that found in the marrow sample taken at the time of diagnosis, which suggests that the original clone of leukemic cells was responsible for relapse. Our results indicate that assessment of rearrangement of the T-cell antigen receptor gene will be valuable in the diagnosis and management of leukemia and can be used to evaluate clonality in T-cell neoplasia.**

The recent isolation of cDNA clones for the mouse and human T-cell antigen receptor has made possible a number of studies regarding the ontogeny of T cells (1–3). The general genomic structure of the T-cell antigen receptor gene is similar to that of the immunoglobulin genes in that there are variable (*V*) regions, diversity (*D*), joining (*J*) regions, and constant (*C*) region elements (4, 5). Similar to immunoglobulin, the T-cell antigen receptor must be able to recognize a vast array of foreign antigens. This similarity between the immunoglobulins and the T-cell antigen receptor with regard to gene structure and protein function suggests that a similar mechanism for generating diversity of the receptor molecule is functional in both B cells and T cells.

In the case of B cells, diversity is achieved in part by somatic rearrangement of the immunoglobulin genes prior to the production of a functional immunoglobulin molecule. Such rearrangements are detected by Southern blot analysis of DNA isolated from B cells. Similar rearrangements have been found in mouse and human T-cell lines when the T-cell antigen receptor gene has been used as a probe (2, 6). In the limited number of studies performed, rearrangement of the T-cell receptor gene has been seen only in T cells; this suggests that somatic rearrangement of the T-cell antigen receptor gene might be a good marker of the T-cell lineage.

Immunoglobulin genes have been used to study the lineage relationship of leukemias and lymphomas (7–11). In this system, detectable somatic rearrangement of the immunoglobulin genes correlated well with the B-cell origin of the malignant cell. We have analyzed DNA isolated from the leukemic cells of patients with acute lymphoblastic leukemia (ALL) to determine whether the T-cell receptor probe would be of similar value. We have found that rearrangement of the T-cell receptor is present in all cases of T-cell leukemia as defined by cell surface phenotyping. In one case identified as a T-cell malignancy based on cell surface markers, we found that both the T-cell antigen receptor β gene and a single allele of the immunoglobulin heavy chain gene were rearranged. These studies indicate that the rearrangement of DNA with respect to the T-cell receptor genes may be useful in the diagnosis and management of certain neoplastic disorders.

MATERIALS AND METHODS

Sources of DNA. DNA was prepared from peripheral blood or bone marrow cells of patients with leukemia, either at the time of diagnosis, or while in remission, or at the time of relapse. DNA prepared from the granulocytes of normal volunteers was used for control studies. DNA was also isolated from cultured skin fibroblasts from some of the patients.

Isolation of DNA. DNA was prepared as described (12). Cells were washed twice in phosphate-buffered saline, resuspended in TNE (10 mM Tris/100 mM NaCl/1 mM EDTA, pH 9.0), and added to an equal volume of TNE containing proteinase K (400 μg/ml) and 1% $NaDodSO_4$. The suspension was incubated 4–16 hr at 37°C. The DNA then was extracted once with phenol/chloroform/isoamyl alcohol and several times with chloroform/isoamyl alcohol and then was precipitated with isopropanol. The DNA was resuspended in 10 mM Tris/1 mM EDTA, pH 7.5. The concentration of DNA was determined by spectroscopy; the A_{260}/A_{280} ratio was 1.8–2.0.

Southern Blot Analysis. DNA was cut with one of the restriction enzymes *Eco*RI, *Bam*HI, and *Hin*dIII according to the recommendations of the supplier. Digested DNA was separated on 0.8% agarose gels and transferred to nitrocellulose paper (13). The filters were hybridized in 0.75 M NaCl/0.075 M sodium citrate, pH 7.0/0.1% $NaDodSO_4$/0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone containing sonicated salmon sperm DNA (100

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Abbreviations: ALL, acute lymphoblastic leukemia; V, variable; D, diversity; J, joining; C, constant; kb, kilobase(s).

Table 1. Cell surface phenotype of peripheral blood cells from patients involved in this study

Patient	Diagnosis	Myeloid*	Antibody							
			Ia	Calla	B1	T_3	T_4	T_6	T_8	T_{11}
1	T-ALL	−	−	−	−	−	+	−	+	+
2	T-ALL	−	−	+	−	+	+	+	+	+
3	T-ALL	−	−	−	−	−	−	−	−	+
4	T-ALL	−	−	−	−	+	−	−	−	−
5	T-ALL	−	−	+	−	+	−	+	−	−
6	T-ALL	−	−	−	−	+	+	+	+	+
7	T-CLL	−	−	−	−	+	−	−	+	+
8	T-CLL	−	−	−	−	+	−	−	−	+
9	T-ALL	−	−	−	−	+	−	+	−	+
10	T-ALL	−	−	−	−	+	+	−	−	−
11	T-ALL	−	−	−	−	+	+	−	−	−
12	T-ALL					+	+			+
13	T-ALL						+	−	+	+
14	T-ALL			+				+		+

A population of cells was considered positive if >30% of the cells stained with the antibody (11, 15).
*Myeloid refers to a panel of antibodies specific for that lineage.

μg/ml) and radioactive probe (5×10^6 cpm/ml). Hybridization was at 65°C for 24 hr. Filters were then washed thoroughly and autoradiographed for various times at −70°C with an intensifying screen.

Probes. Two probes were used in these experiments. One was a *Bgl* II/*Eco*RV fragment of the cDNA clone YT35 (1) that contained only the *C* region of the T-cell receptor gene. The other probe was a *Hin*dII/*Eco*RV fragment that contained the *C* region and the *J* region. The probe was made radioactive by nick-translation with [α-^{32}P]dCTP (14).

Cell-Surface Phenotype. A panel of monoclonal antibodies was used to study the leukemic cells (11, 15). The phenotypes of the leukemic cells are shown in Table 1.

RESULTS

Determination of Somatic Rearrangement. To determine whether the T-cell receptor gene has undergone somatic rearrangement, DNAs from patients with various forms of leukemia were studied. The first studies involved paired samples of DNA from two patients with T-cell ALL. One of each pair of samples was DNA from the patient's skin fibroblasts, whereas the second DNA sample was derived from the patient's peripheral blood cells (>95% blast cells). We assume that the fibroblast DNA contains the T-cell receptor gene in its germ-line configuration. The results of Southern blots of these DNAs are shown in Fig. 1. The DNA was cut with either *Eco*RI, *Bam*HI, or *Hin*dIII and probed with the *Hin*dII/*Eco*RV fragment of YT35 (1). In each case, the peripheral blood cell DNA showed a pattern that was different from the germ-line fibroblast DNA. The germ-line pattern generated by each of these enzymes is quite distinct. *Eco*RI gave three germ-line bands of 11.1 kilobases (kb), 9.5 kb, and 4.3 kb. *Bam*HI gives a single germ-line band of 22 kb. The germ-line configuration digested by *Hin*dIII gives a series of bands of 12 kb, 8 kb, 6.5 kb, and 3.5 kb. Regardless of which of these three enzymes was used, differences indicative of somatic rearrangement can be seen between the peripheral blood cell and fibroblast DNA. Note that in *Eco*RI and *Bam*HI digests of peripheral blood cell DNA, bands consistent with the germ-line pattern are absent. The potential significance of this is discussed below.

The above experiment indicated that somatic rearrangement does occur in leukemic T cells. To determine whether polymorphism in the T-cell receptor gene is common, we studied DNA from 23 normal individuals. The DNA was cut with the restriction enzyme *Hin*dIII or *Eco*RI and probed with the *Bgl* II/*Eco*RV fragment of the T-cell antigen receptor gene. In all cases the same pattern was seen; furthermore, this pattern was the same pattern as that seen for the fibroblast DNA from the two patients with T-cell ALL. These results indicate that the T-cell receptor gene is not highly polymorphic in the general population. Fig. 2 shows the pattern observed for granulocyte DNA from normal individuals. The germ-line pattern produced by *Eco*RI (Fig. 2*a*) differs from the fibroblast germ-line *Eco*RI pattern (Fig. 1) in

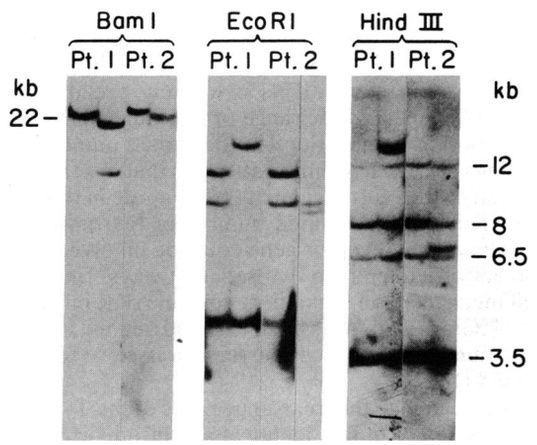

FIG. 1. DNA from two patients (Pt. 1 and 2) with T-cell ALL; the DNA in the left lane of each pair was derived from skin fibroblasts whereas the DNA in each right lane was derived from peripheral blood leukemic cells. DNA was digested with the indicated restriction enzyme, separated on a 0.8% agarose gel, and transferred to nitrocellulose. The filter was probed with the *Hin*dII/*Eco*RV fragment of YT35 and autoradiographed.

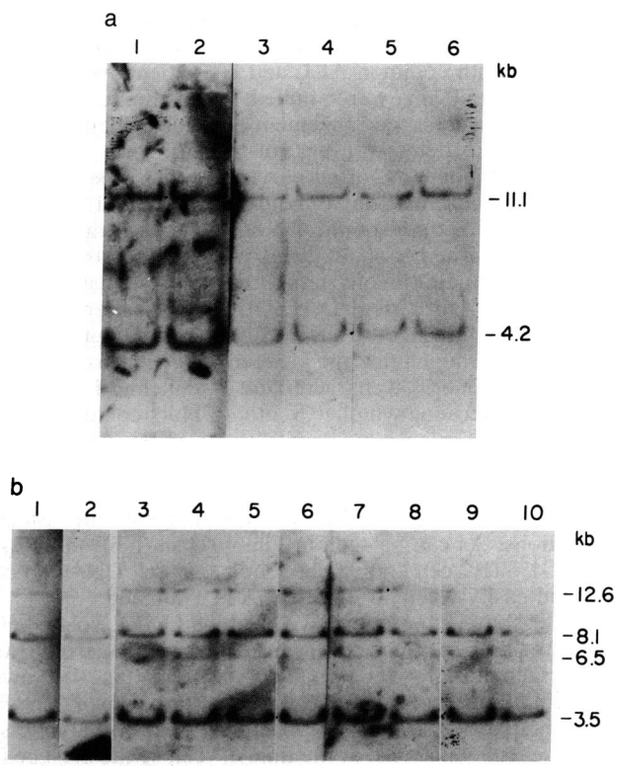

FIG. 2. Southern blot of granulocyte DNA obtained from normal individuals and digested with either *Eco*RI (*a*) or *Hin*dIII (*b*). The digested DNA was electrophoresed, transferred to nitrocellulose, and probed with the *Bgl* II/*Eco*RV fragment of YT35. Numbers above each lane refer to the same individuals in both *a* and *b*.

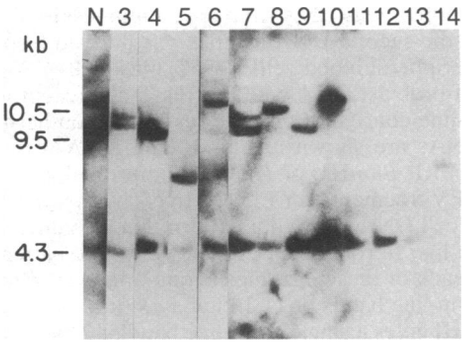

FIG. 3. Granulocyte DNA from a normal individual (lane N) and DNAs from leukemic cells (bone marrow or peripheral blood) from 12 patients with T-cell leukemia (lane numbers correspond to patient numbers in Table 1) were cut with *Eco*RI, separated on a 0.8% gel, and transferred to nitrocellulose. The filter was probed with the ^{32}P-labeled *Hin*dII/*Eco*RV fragment of YT35.

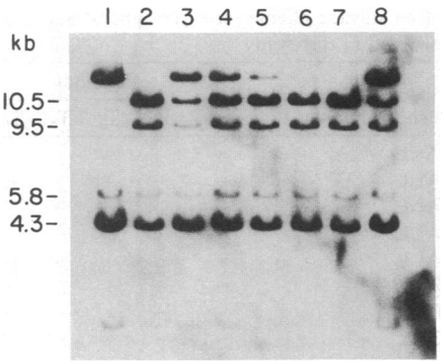

FIG. 4. This blot illustrates the use of analysis of the T-cell antigen receptor gene in following the course of a patient. DNA from patient 1 (Table 1) was used in these studies. The DNA was cut with *Eco*RI, electrophoresed, transferred, and then probed with the *Hin*dII/*Eco*RV fragment. Lane 1, bone marrow DNA obtained at the time of diagnosis; 90% of the cells were of blast form. Lane 2, fibroblast DNA. Lanes 3–6; DNA samples (10 μg) composed of the bone marrow cell DNA sample and the fibroblast DNA sample mixed in a 1:1 (lane 3), 1:4 (lane 4), 1:19 (lane 5), or 1:100 (lane 6) ratio (wt/wt). Lane 7, bone marrow DNA obtained when the patient was in remission (<5% blast cells in the bone marrow). Lane 8, bone marrow DNA obtained at relapse (70% of the bone marrow cells were blast cells).

that the 9.5-kb band seen in the latter is missing from the former. This difference is attributable to the use of the *Bgl* II/*Eco*RV probe (Fig. 2).

Survey of DNAs from Leukemic Cells. Having found that the *Eco*RI pattern is conserved between individuals, we used this enzyme to digest DNA derived from the leukemic cells of 12 patients with T-cell leukemia as defined by cell-surface markers. The results are shown in Fig. 3. As can be seen, the pattern of the T-cell antigen receptor gene in these patients is different from the germ-line pattern. The pattern of rearrangement observed in these 14 cases appears to be limited in that samples from different individuals appear to bear the same rearrangements; for example, patients 3 and 7 and patients 4 and 9 have similar rearrangements.

Specificity of T-Cell Antigen Receptor Rearrangement. To determine whether rearrangement of the T-cell antigen receptor gene occurs in other forms of leukemia, DNA from six patients with common ALL and six patients with acute myeloblastic leukemia were studied. In this limited number of cases, no somatic rearrangement of the T-cell antigen receptor gene was detected (data not shown).

Patient 4 has been reported previously (11). The cell-surface phenotype of this patient indicated a T-cell ALL. However, analysis of the genomic DNA showed rearrangement of one of the μ heavy chain genes whereas the other allele was still in the germ-line form (11). In the present study, we show that DNA of leukemic cells from this patient is also rearranged with respect to the T-cell antigen receptor gene.

Having determined that the presence of genetic rearrangement was, in general, a characteristic of leukemic T cells, we wanted to determine whether Southern blots could be used to distinguish a relapse marrow from a remission or normal marrow. To do this, we performed two different experiments. In the first, a patient's leukemic (bone marrow) DNA and normal (fibroblast) DNA were mixed together in various proportions. As can be seen in Fig. 4 (lanes 1–6), the presence of 1% (10^4 cell equivalents) leukemic cell DNA can be detected easily when 10 μg of DNA is examined.

In the second experiment, we examined DNA isolated either at the time of relapse or from a "remission" marrow. As can be seen in Fig. 4, the remission specimen (lane 7) shows the germ-line pattern, whereas the relapse specimen (lane 8) shows both the germ-line pattern and the pattern characteristic of the leukemic cells; at the time of relapse the blast cells represented ≈70% of the marrow cells. In addition, the rearrangement pattern of the DNA during relapse is similar to that of the bone marrow DNA isolated at the time of diagnosis (lane 1), indicating that the same clone of leukemic cells emerged upon relapse.

DISCUSSION

In this paper, we have demonstrated that the T-cell receptor gene has undergone genetic rearrangement in T cells isolated from T-cell ALL patients. This finding is of importance for several reasons. First, it shows that the malignant T cells are clonal in nature. Furthermore, the finding that DNA from the relapse marrow contains the same rearrangement as existed in the presentation sample of leukemic cells indicates that, in this patient, the same clone of leukemic cells was responsible for leukemic relapse.

Examination of the patterns of rearrangement indicates that the rearrangements are not totally random and suggests that a limited number of patterns may be found in T-cell ALL leukemic cells. For example, in six samples, four different patterns are seen; patients 3 and 7 and patients 4 and 9 have similar patterns. In addition, we have previously reported that the patterns in MOLT-3 and Jurkat cells are similar (6). The finding of a number of different patterns is consistent with the presence of a number of *V*, *D*, and *J* gene segments present in the T-antigen receptor gene locus. The limited number of patterns may be due to a small number of possible rearrangements. This is unlikely because in functional T-cell clones a wide range of rearrangements are seen (6, 16). Another explanation for the limited number of rearrangements seen in leukemic patients is that in some way the generation of the rearrangements plays a role in the development of leukemia. A limited number of rearrangements of the T-cell antigen receptor gene could be involved in the development of leukemia in two separate ways: first, the postulated mechanism of leukemogenesis in AKR mice presented by McGrath and Weissman (17) and second, the finding that the human T-cell receptor β chain is close to the oncogene *erb8* (18).

Further evidence that rearrangements of the T-cell antigen receptor gene are in fact identical and of importance in leukemia is needed. This may be obtained through the use of other restriction endonucleases, the study of a larger number of patients, and the isolation of the rearranged fragments. It is significant that the complete nucleotide sequences of the β chain message of Jurkat and MOLT-3 cells are identical (19).

Rearrangement is present in all of the leukemic cells, which suggests that the development of the malignant pheno-

type occurred at or after the time of rearrangement.

At the present time some information is available for the genomic structure of the T-cell receptor gene. It has been determined that there is a 5′ region of *V* sequences followed by a series of *D* and *J* genes and a *C* region. Located 3′ to this is a second set of *D* and *J* sequences and a second *C* region; the first *D*, *J*, and *C* are referred to as *DB1*, *JB1*, and *CB1*, whereas the second set are referred to as *DB2*, *JB2*, and *CB2* (5). In the experiments reported here two probes were used: one (*Bgl* II/*Eco*RV fragment) that contained the *C* region and 3′ untranslated region, and a second (*Hin*dII/*Eco*RV fragment) that contained the *C* region, 3′ untranslated region, and part of the *J* region. The different probes account for the differences seen in the germ-line pattern after *Eco*RI digestion. The 8.5-kb band seen with the *Hin*dII/*Eco*RV fragment is not seen with the *Bgl* II/*Eco*RV fragment. Thus, this band contains the *J* genes. The 4.3-kb band, seen with either of these probes after *Eco*RI digestion, is present in each of the cases studied. This suggests that this band contains the 3′ untranslated sequences and represents DNA 3′ to the rearranged DNA.

The pattern of rearrangement seen with either *Eco*RI or *Bam*HI is intriguing. As already mentioned there are two alleles of each of the germ-line *C* regions. However, in none of the cases in which there has been gene rearrangement is there persistence of the germ-line pattern. This suggests that, as in the case of the immunoglobulin genes, there is deletion of noncoding genes (4) or rearrangement of both alleles. With the use of the restriction enzyme *Hin*dIII, somatic rearrangements can be detected in the DNA of some patients. As there is a *Hin*dIII site just 3′ to *JB1* (20), rearrangements detected with this enzyme are likely to have occurred into *CB2*. To determine the possible sequence of rearrangement, further studies with defined probes for the different *J* and *D* gene segments are needed.

The finding that rearrangement of this gene has occurred in cells considered to be of T-cell lineage further confirms the hypothesis that this gene is the T-cell antigen receptor gene and that rearrangement of this gene is a characteristic of T cells. The finding of a case in which both the immunoglobulin and T-cell genes have rearranged is an exception to the rule and raises the question of the specificity of genetic rearrangement in determining or defining cell lineage. As shown here and in the case of B cells and immunoglobulin genes, genetic rearrangement of an antigen receptor gene is necessary for the development of a mature and functional cell; however, the presence of a rearrangement does not restrict a cell to a single differentiation pathway. Alternatively, the finding of genetic rearrangement may be another manifestation of lineage infidelity in malignant cells (21).

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