α-Chain locus of the T-cell antigen receptor is involved in the t(10;14) chromosome translocation of T-cell acute lymphocytic leukemia

(genetic recombination/oncogene activation/*TCL3* gene/genetics of leukemia)

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Contributed by Peter C. Nowell, March 18, 1987

ABSTRACT Human leukemic T cells carrying a t(10;14)(q24;q11) chromosome translocation were fused with mouse leukemic T cells, and the hybrids were examined for genetic markers of human chromosomes 10 and 14. Hybrids containing the human 10q+ chromosome had the human genes for terminal deoxynucleotidyltransferase that has been mapped at 10q23–q25 and for C_α [the constant region of *TCRA* (the α-chain locus of the T-cell antigen receptor gene)], but not for V_α (the variable region of *TCRA*). Hybrids containing the human 14q− chromosome retained the V_α genes. Thus the 14q11 breakpoint in the t(10;14) chromosome translocation directly involves *TCRA*, splitting the locus in a region between the V_α and the C_α genes. These results suggest that the translocation of the C_α locus to a putative cellular protooncogene located proximal to the breakpoint at 10q24, for which we propose the name *TCL3*, results in its deregulation, leading to T-cell leukemia. Since hybrids with the 10q+ chromosome also retained the human terminal deoxynucleotidyltransferase gene, it is further concluded that the terminal deoxynucleotidyltransferase locus is proximal to the *TCL3* gene, at band 10q23–q24.

Human T-cell leukemias and lymphomas frequently show specific chromosome rearrangements, predominantly translocations and inversions (1, 2), involving chromosome region 14q11, the site of the α-chain locus of the T-cell antigen receptor (*TCRA*) (3). The direct involvement of *TCRA* in the t(11;14)(p13;q11) and the t(8;14)(q24;q11) translocations observed in acute T-cell leukemias was proven by the somatic cell genetic analysis of leukemic cells (4, 5) and by the molecular analysis of the breakpoints involved in the translocations (6). Finger *et al.* (6) also showed that the t(8;14)(q24;q11) translocation results in the association of a region on the 3' side (distal) of the *MYC* oncogene at 8q24 with one of the loci for the joining region of the *TCRA* (J_α), and that the translocation is catalyzed by the same enzymatic system involved in *TCR* gene rearrangements. The juxtaposition of the *MYC* locus and of the *TCRA* results in deregulation of the *MYC* gene involved in the translocation, leading to neoplasia (5).

A t(10;14)(q24;q11) chromosome translocation has been detected in acute T-cell leukemia and in high grade T-cell lymphomas (2, 7, 8). We have analyzed cells from a patient with acute T-cell leukemia who possessed this translocation and have asked whether the t(10;14) chromosome translocation directly involves the *TCRA*. If so, it would seem likely that a cellular protooncogene located at 10q24 might be deregulated by mechanisms analogous to those in other T-cell neoplasms with chromosomal translocations involving the *TCRA*.

MATERIALS AND METHODS

Patient. The patient (DW) is a 23-year-old man who was admitted to the hospital with a 2-month history of headaches, fever, weakness, shortness of breath, weight loss, and bleeding from a recent dental extraction. He had had a splenectomy 7 years earlier for traumatic injury and was known to have sickle cell trait. He had a slight fever and palpable lymph nodes (1–2 cm) in cervical, axillary, and groin areas. His blood counts revealed a severe anemia with a hematocrit of 19%, thrombocytopenia of 40,000 platelets per mm^3, and an elevated leukocyte count of 147,000 leukocytes per mm^3, predominantly lymphocytes (73%) with 25% lymphoblasts. On chest x-ray and computerized tomography scan, there was a large anterior mediastinal mass extending into the subcarinal region and the pericardium. The bone marrow was 95% cellular, with a predominance of lymphocytic blast cells containing convoluted nuclei. An axillary lymph node biopsy revealed a completely effaced architecture with subcapsular infiltration by neoplastic cells. The immunophenotype of the circulating lymphocytes was: $T11^+$, $T6^+$, $T4^-$, $T8^+$, SIg^-, $CALLA^+$, TdT^+, Ia^-. A diagnosis of T-cell lymphoblastic leukemia/lymphoma was established, and chemotherapy was initiated using the L10/17 protocol (9). The patient entered complete remission after induction, had a difficult consolidation, and is currently receiving maintenance therapy. Leukemic cells for study were obtained from the peripheral blood prior to treatment.

Cells. DNA was extracted from the leukemic cells (DW) for Southern blot analysis (4, 5). The leukemic cells were also fused with mouse leukemic BW5147 cells, deficient in hypoxanthine phosphoribosyltransferase, using polyethylene glycol, and hybrids were selected in hypoxanthine/aminopterine/thymidine (HAT) medium according to standard procedures (4, 5). Independent hybrids were selected and then cultures were expanded.

Chromosome Analysis. Chromosome preparations were made from peripheral blood leukocytes cultured for 24 hr without mitogen. They were air-dried and banded by the trypsin/Giemsa banding method (10). At least 25 metaphases were examined for each hybrid, and they were scored only when the quality of the banding and staining of human chromosomes was adequate. Selected metaphases were studied by the G11 banding technique to confirm the human origin of the relevant chromosomes (10).

Abbreviations: TdT, terminal deoxynucleotidyltransferase; *TCRA*, α-chain locus for the T-cell antigen receptor gene; J_α, V_α, and C_α, joining, variable, and constant regions, respectively, of *TCRA*.

Isozyme Analysis. Hybrids were studied for the expression of nucleoside phosphorylase, the gene for which is proximal to *TCRA* on chromosome 14 by starch gel electrophoresis (4, 5).

DNA Gel Electrophoresis. Agarose gel (0.7% or 1%) electrophoresis was carried out in 40 mM Tris·HCl/5 mM NaOAc/2.0 mM EDTA, pH 8.8. *Hin*dIII-digested phage DNA molecular weight markers (0.75 μg per lane) (Bethesda Research Laboratories) were included on every gel. DNA samples were cleaved with restriction enzymes and then subjected to electrophoresis in a horizontal agarose (Sigma) slab gel (10 μg of DNA per lane). Gels were stained for 10 min with ethidium bromide (1 μg/ml) and photographed under UV light. Transfer of DNA from gel to nitrocellulose sheet (Millipore) was performed essentially as described by Southern (11).

Preparation of Labeled Probes. DNA probes for the variable region of *TCRA* (V_α) (*Ava* I–*Pst* I fragment isolated from pHαT3 cDNA clone) (3) and for the constant region of *TCRA* (C_α) (pHTα2) (3), for the immunoglobulin heavy-chain joining region (pHj) (12, 13), and for the enzyme terminal deoxynucleotidyltransferase (TdT) (pT223; a full-length TdT cDNA clone) (14) were used to detect the corresponding genes in the hybrid clones. The probes were labeled with [α-^{32}P]NTP (from Amersham) by the nick-translation procedure (15). Labeled probes with specific activities of 0.5–5 × 10^8 cpm/μg of DNA were used.

Hybridization. DNA on nitrocellulose sheet was hybridized to ^{32}P-labeled probe DNAs (V_α, C_α, immunoglobulin heavy-chain joining region, and TdT) in a hybridization solution containing 50% (vol/vol) formamide (4, 5). After hybridization the filters were washed, air dried, and exposed to Kodak XAR-5 film for various periods. Prior to hybridization, filters were washed two times at 65°C with H$_2$O for 15 min (4, 5).

RESULTS

Chromosome and Isozyme Analysis of Hybrid Clones. The karyotype of the parental DW leukemic cells is shown in Fig. 1. The only abnormality is a t(10;14)(q24;q11) chromosome translocation, apparently identical to those reported in T-cell acute lymphocytic leukemias and high-grade lymphomas (2, 7, 8).

The results of the karyotype analysis of hybrid clones (Fig. 2) from fusions between leukemic cells and mouse BW5147 cells are shown in Table 1. Hybrids 639 AD-4 and 639 CB-6
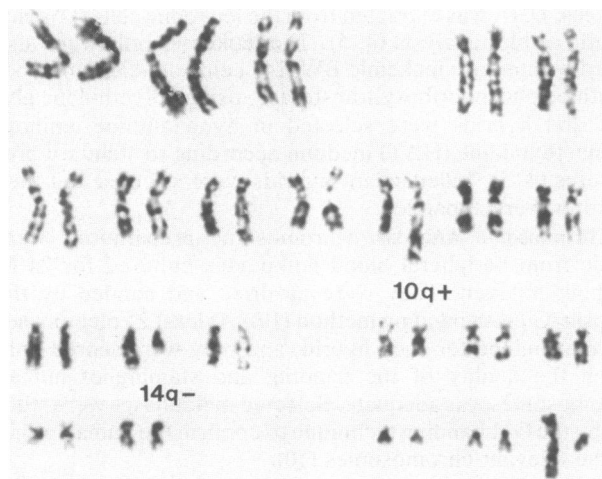

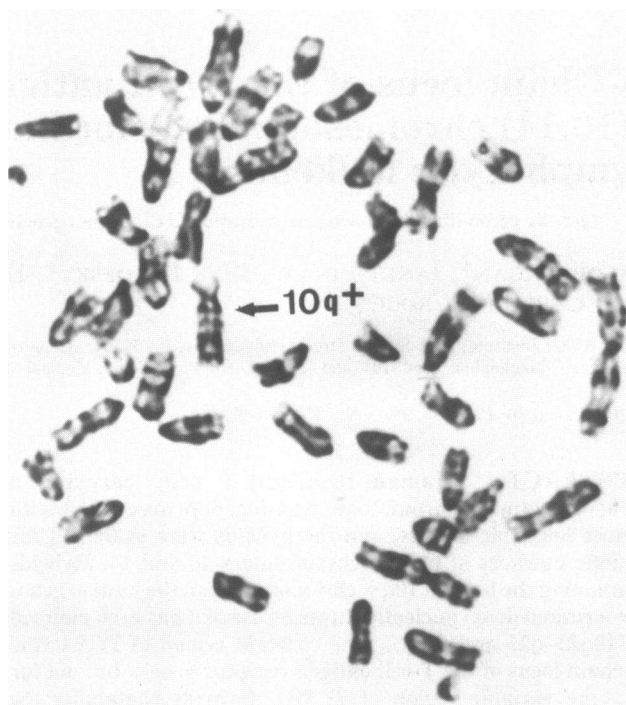FIG. 1. Karyotype of DW leukemic cells, 46 XY, t(10;14)(q24;q11).

FIG. 2. Partial G-banded metaphase of hybrid 639 AD-4 that contained the 10q+ (arrow) but not the normal 10, normal 14, or 14q− chromosomes.

contained only the 10q+ chromosome, having lost the other three relevant human chromosomes, 10, 14, and 14q−. Hybrid 639CC-2 retained both the 10q+ and the 14q− chromosomes. Analysis of the hybrids for expression of human nucleotide phosphorylase indicated that the breakpoint on chromosome 14 at band q11 was distal to the nucleotide phosphorylase gene, since hybrids with only the 10q+ chromosome were negative for human nucleotide phosphorylase (639 AD4 and 639 CB6), while hybrid 639 CC-2 that contained the 10q+ and the 14q− chromosomes was positive for human nucleotide phosphorylase (Table 1).

Southern Blot Analysis of Hybrid DNAs. As shown in Fig. 3C and Table 1, hybrids 639 AD-4 and 639 CB-6 containing the 10q+ human chromosome in the absence of the other relevant human chromosome had the human TdT gene. Hybrid 639 DC-2, containing the 14q− chromosome in the absence of the 10q+ and of the normal chromosome 10, was negative for TdT. These results indicate that the TdT gene is proximal to the breakpoint on chromosome 10. Presence of the human immunoglobulin heavy-chain joining region, located at band 14q32, was detected only in hybrids containing either the chromosome 10q+ (639 AD-4 and 639 CB-6) or the normal chromosome 14 (639 DC-2) as expected (Table 1).

Table 1. Human genes in DW–BW5147 hybrids

Cell line	Human genes					Human chromosomes			
	V_α	C_α	NP	J_H	TdT	10	10q+	14	14q−
DW	+	+	+	+	+	+	+	+	+
CMC 639 AD-4	−	+	−	+	+	−	++	−	−
CMC 639 CB-6	−	+	−	+	+	−	++	−	−
CMC 639 CC-2	+	+	+	+	+	−	++	−	++
CMC 639 DC-2	+	+	+	+	−	−	−	+	±
CMC 639 DA-2	−	−	−	−	−	−	−	−	−
CMC 639 CD-5	−	−	−	−	−	−	−	−	−

Frequency of metaphases with relevant human chromosomes: −, none; ±, <10%; +, 10–30%; ++, >30%; NP, nucleotide phosphorylase; J_H, immunoglobulin heavy-chain joining region.
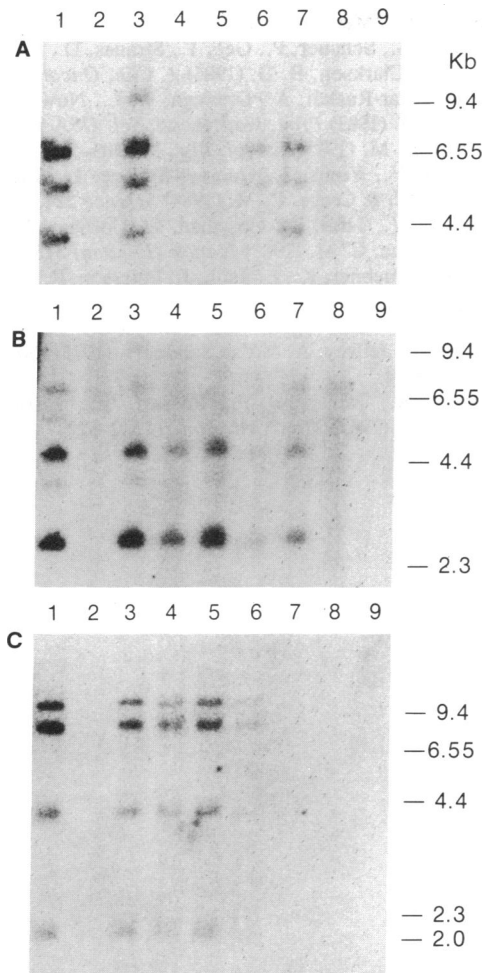
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FIG. 3. Southern blot analysis of hybrid clones. Hybridization of *Hind*III-digested cellular DNA with V_α-specific probe (*A*), C_α-specific probe (*B*), and TdT-specific probe (*C*). Lanes: 1, GM1500 human lymphoblastoid cell line DNA; 2, parental mouse BW5147 leukemic cell DNA; 3, parental DW DNA; 4, hybrid 639 AD-4 DNA; 5, hybrid 639 CB-6 DNA; 6, hybrid 639 CC-2 DNA; 7, hybrid 639 DC-2 DNA; 8, hybrid 639 DA-2 DNA; 9, hybrid 639 CD-5 DNA. Sizes are in kilobases (Kb).

The hybrids were then studied for the V_α and C_α. As shown in Fig. 3*B*, hybrids 639 AD-4 and 639 CB-6, which contained only the 10q+ chromosome (Table 1), had the C_α gene (Fig. 3*B*) but not the V_α genes (Fig. 3*A*). Hybrid 639 CC-2, which retained both the 10q+ and the 14q− chromosomes, had both C_α and V_α genes (Fig. 3 *A* and *B*; Table 1). These results indicate that *TCRA* is split by the chromosomal breakpoint in a region between C_α and the V_α genes (Fig. 4), with the C_α segment translocated to chromosome 10.

No rearrangements of *TCRA* (Fig. 3*B*) in the DW leukemic cells were detected using a C_α probe presumably because the α locus contains many J_α segments spanning more than 100 kilobases on the 5′ side of the C_α locus, and the C_α region may have been some distance from the breakpoint.

DISCUSSION

These results of the analysis of somatic cell hybrids between mouse leukemic T cells and human leukemic T cells carrying a t(10;14)(q24;q11) chromosome translocation indicate that the breakpoint on chromosome 10 is distal to the TdT gene and helps to localize the gene to region 10q23−q24. The results also indicate that the breakpoint on chromosome 14 involves directly *TCRA* in the region between the C_α gene and the V_α genes. It has been shown (6) that in T-cell leukemias
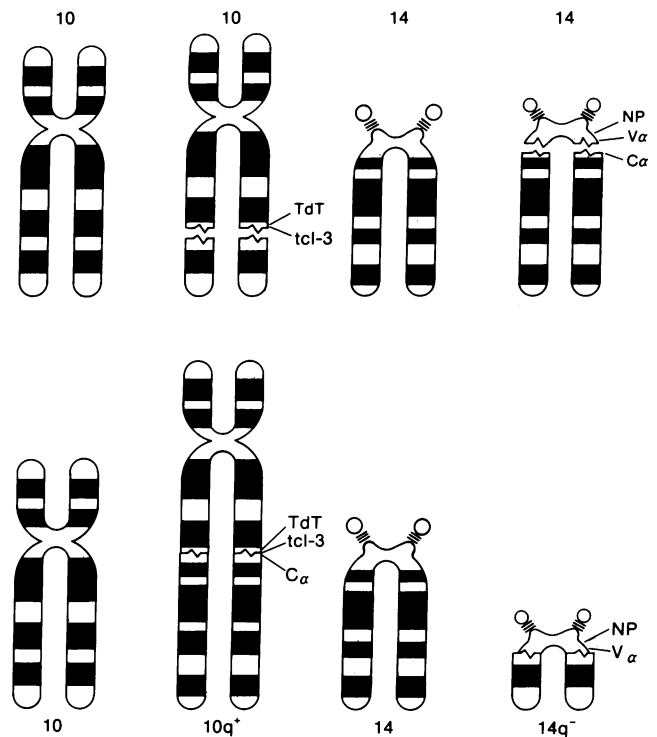

FIG. 4. Diagram of the t(10;14)(q24;q11) chromosome translocation of acute T-cell leukemia. The *TCRA* is split by the chromosome 14 breakpoint between the C_α and the V_α genes. The C_α locus of *TCRA* translocates to a region of chromosome 10 at band q24, where the putative *TCL3* (tcl-3) gene resides, leading to *TCL3* activation and to T-cell neoplasia.

with a t(8;14)(q24;q11) translocation the breakpoint on chromosome 14 directly involves a J_α segment ≈36 kilobases on the 5′ side of the C_α gene (6). Thus, it seems likely that J_α segments may also be involved in the t(10;14) chromosome translocation. Cloning and sequencing of the breakpoint should indicate whether this is the case and whether this t(10;14) chromosome translocation is also catalyzed by the recombinase involved in physiologic TCR gene rearrangements (6).

The fact that the C_α locus is translocated to a region of chromosome 10 that is involved in translocations in ≈10% of acute T-cell leukemias (8) suggests quite strongly that on chromosome 10 at band q24, distal to the TdT gene, there is a gene involved in T-cell proliferation, the deregulation of which leads to a high-grade T-cell malignancy (Fig. 4). We propose the name of *TCL3* (T-cell leukemia/lymphoma 3) for this gene. Since the human genes for glutamine oxaloacetic transaminase (*GOT*) and for TdT map to region 10q25–q26 and 10q23–q24, respectively, and the homologous genes of the mouse map to mouse chromosome 19, it appears that the region involved in the t(10;14) chromosome translocation in man is homologous to a region of mouse chromosome 19. It is of considerable interest that Villemur *et al.* (16) have found that murine leukemia virus integrates into a specific region of mouse chromosome 19 in ≈25% of virus-induced T-cell leukemias that they have analyzed. Thus it seems possible that the putative *TCL3* gene, which is activated by the translocation of the *TCR* gene in man, might be activated by retroviral insertion in the mouse.

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