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/TCR3 gene/genetics of leukemia)

JACOB KAGAN, JANET FINAN, JEAN LETOFISKY, EMMANUEL C. BESA, PETER C. NOWELL, and CARLO M. CROCE

*The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104; †Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104; and ‡Department of Medicine, Medical College of Pennsylvania, Philadelphia, PA 19129

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ABSTRACT Human leukaemic T cells carrying a t(10;14)(q23;q11) chromosome translocation were fused with mouse leukaemic T cells, and the hybrids were examined for genetic markers of human chromosones 10 and 14. Hybrids containing the human 10q+ chromosome had the human genes for terminal deoxynucleotidyl transferase that has been mapped to 10q23–q25 and for Cα (the constant region of TCRα (the α-chain locus of the T-cell antigen receptor gene)), but not for Vα (the variable region of TCRα). Hybrids containing the human 14q+ chromosome retained the Vα genes. These results suggest that the translocation of the Cα locus to a putative cellular protooncogene located proximal to the breakpoint at 10q24, for which we propose the name TCI3, results in its deregulation, leading to T-cell leukaemia. Since hybrids with the 10q+ chromosome also retained the human terminal deoxynucleotidyl transferase gene, it is further concluded that the terminal deoxynucleotidyl transferase locus is proximal to the TCI3 gene, at band 10q23–q24.

Human T-cell leukaemias 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)(q13;q11) and the t(8;14)(q24;q11) translocations observed in acute T-cell leukaemias was proven by the somatic cell genetic analysis of leukaemic 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 (7), 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)(q23;q11) chromosome translocation has been detected in acute T-cell leukaemia and in high grade T-cell lymphomas (2, 7, 8). We have analyzed cells from a patient with acute T-cell leukaemia 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 mm³, and an elevated leukocyte count of 147,000 leukocytes per mm³, 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 auxiliary lymph node biopsy revealed a completely effaced architecture with subcapsular infiltration by neoplastic cells. The immunophenotype of the circulating lymphocytes was: Tc1, Tc6, Tc4, Tc8, SAg, CALLA, TcD, 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 leukaemic cells (DW) for Southern blot analysis (4, 5). The leukaemic cells were also fused with mouse leukaemic BW5147 cells, deficient in hypoxanthine phosphoribosyltransferase, using polyethylene glycol, and hybrids were selected in hypoxanthine/aminopterin/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 deoxynucleotidyl transferase; TCRA, α-chain locus for the T-cell antigen receptor gene; Vα, Vα and Cα, joining, variable, and constant regions, respectively, of TCRA.

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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. HindIII-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 (Yα) (Avt I-PstI fragment isolated from phHT3 cDNA clone) (3) and for the constant region of TCRA (Cγ) (pHT225, a full-length TdT-cDNA clone) (14) were used to detect the corresponding genes in the hybrid clones. The probes were labeled with [α-32P]dCTP (from Amersham) by the nick-translaction procedure (15). Labeled probes with specific activities of 0.5–5 × 106 cpm/μg of DNA were used.

Hybridization. DNA on nitrocellulose sheet was hybridized to 32P-labeled probe DNAs (Yα, Cγ, 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 H2O 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 BWS147 cells are shown in Table 1. Hybrids 639 AD-4 and 639 CB-6 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 Blast 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-BWS147 hybrids

<table>
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<th>Human genes</th>
<th>DW</th>
<th>CMC 639 AD-4</th>
<th>CMC 639 CB-6</th>
<th>CMC 639 CC-2</th>
<th>CMC 639 DC-2</th>
<th>CMC 639 DA-2</th>
<th>CMC 639 CD-5</th>
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</table>

Frequency of metaphases with relevant human chromosomes: none; +, <10%; +, 10–30%; ++, >30%; NP, nucleotide phosphorylase; JαH, immunoglobulin heavy-chain joining region.
**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 10q22-23. The results also indicate that the breakpoint on chromosome 14 involves directly TCRα in the region between the Cα gene and the Vα genes. It has been shown (6) that in T-cell leukemias with a t(8;14)(q24;q31) translocation the breakpoint on chromosome 14 directly involves a Jα segment ~36 kilobases on the 5' side of the Cα gene (6). Thus, it seems likely that Jα 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 recombination involved in physiologic TCR gene rearrangements (6).

The fact that the Cα 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 oxaloacetate transaminase (GOT) and for TdT map to region 10q25-26 and 10q23-24, 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 Villenur 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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1. Zech, L., Gartho, G., Hammerstrom, J., Julissasco, G.,
