Molecular analysis of a t(14;14) translocation in leukemic T-cells of an ataxia telangiectasia patient
(chromosome translocation/TCL1/oncogene activation/human leukemia)

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ABSTRACT We have detected and cloned two rearrangements in the T-cell receptor Jα locus from a clone of somatic cell hybrids carrying a t(14;14)(q11;q32) chromosomal translocation derived from an ataxia telangiectasia patient with T-cell chronic lymphocytic leukemia. The T-cell clone carrying the t(14;14) chromosomal translocation was known to be present for >10 years before the onset of overt leukemia. One molecular rearrangement of the T-cell receptor Jα locus corresponded to a functional variable–joining region (V-J) joining, whereas the other derived from the breakpoint of the t(14;14)(q11;q32) translocation. Chromosomal in situ hybridization of the probe derived from the t(14;14) breakpoint localized the breakpoint region to 14q32.1, apparently the same region that is involved in another ataxia telangiectasia characteristic chromosome translocation, t(7;14)(q35;q32). The 14q32.1 breakpoint is at least 10,000 kilobase pairs (kb) centromeric to the immunoglobulin heavy chain locus. Sequence analysis of the breakpoint indicates the involvement of a Jα sequence during the translocation. Cimigration of high-molecular weight DNA fragments involved with t(7;14) and t(14;14) translocations suggests the presence of a cluster of breakpoints in the 14q32.1 region, the site of a putative oncogene, TCL1.

Many malignancies of the hematopoietic lineage exhibit nonrandom chromosomal translocations (1–3). B-cell tumors are frequently associated with the t(8;14)(q24;q32) translocation in Burkitt lymphomas, with the t(14;18)(q21;q23) translocation in follicular lymphoma, and with the t(11;14)(q13;q32) translocation in B-cell chronic lymphocytic leukemia and sporadically in multiple myeloma and some non-Hodgkin lymphomas (4, 5). Although lymphocytic neoplasms of T-cell origin are considerably less common than their B-cell counterparts, chromosomal translocations and inversions are also present in many of these cases. In both B- and T-cell tumors, immunoglobulin and T-cell receptor (TCR) genes, respectively, are involved in these translocations, and oncogenes or putative oncogenes loci are nonrandomly altered and/or activated during the translocation event (5). Loci that together with the TCR genes are frequently involved in T-cell leukemias map at bands 14q32.1, 11p13, and 10q24 (3, 6–9).

In ataxia telangiectasia (AT), an syndrome characterized by cerebellar ataxia, variable levels of T-cell immunodeficiency, and hypersensitivity to ionizing radiation, both nonrandom chromosomal translocations and lymphoid neoplasms are frequent events (10). Furthermore, preleukemic clonal expansions of cells carrying specific chromosomal abnormalities, such as the inv(14)(q11;q32) inversion or the t(14;14)(q11;q32) translocation, are often observed in these patients (2, 11).

The AT case herein characterized at the molecular level has been widely studied in the past 20 years (1, 2, 12, 13). The patient, a 48-year-old woman with AT, developed a leukemic clone carrying a t(14;14)(q11;q32) chromosomal translocation and multiple additional abnormalities in 1977. Ten years previously, a clinically nonneoplastic clone with only the t(14;14) rearrangement had been demonstrated in her circulating T cells (2). To identify loci involved in the leukemogenic process, we undertook molecular analysis of this chromosome translocation in the leukemic T cells of this patient.

MATERIALS AND METHODS

Somatic Cell Hybrids. Mouse BW5147 T-cell lymphoma cells were fused with the patient’s T lymphocytes as described (14). A hybrid, 513AQB10 (Fig. 1) was isolated that carried the 14q+ (14pter→14q32::14q11→14q32) derived from the t(14;14)(q11;q32) translocation observed in preleukemic and leukemic cells of the patient.

Hybrid 5263C17 is a rodent–human hybrid that carries a t(X;14)(q13;q32) chromosome translocation (15). Hybrid M44C12S9 is a hybrid between mouse myeloma (NP3) and an endemic Burkitt lymphoma (P3HR-1) containing only the 14q+ chromosome derived from the t(8;14)(q24;q32) (16). Hybrid S88-AA3 contains the 8q+ chromosome (8pter→8q34:14q11→14qter) resulting from the translocation that joins a region containing all of chromosome 14 telomeric to a region >36 kilobases (kb) 5′ to the constant (C) region of the TCR chain locus (14q11→14qter) to a region 3′ of c-myc on chromosome 8 (17). No normal chromosome 14 is observed in the parental cell line or in hybrid S88-AA3 (18). These hybrids were used for regional chromosomal localization. Other hybrid cell lines used are briefly described in the text and in figure legends.

Southern Transfer Hybridizations, Molecular Cloning, and Sequencing. The probes mMI12B2.3 and pMI11 detect the TCR Jα segment at 62 and 58 kilobase pairs (kb) from the C region, respectively (M.I. and C.M.C., unpublished results).

Southern transfer hybridization procedures, genomic cloning in the A phage vector EMBL3, and nucleotide sequence analysis using the Sanger dideoxynucleotide chain-termination method on M13-cloned single-strand DNA have been described (19). Sequences were analyzed using the University of Wisconsin Computer Group Software (20).

Abbreviations: TCR, T-cell receptor; AT, ataxia telangiectasia; Jα, joining; V, variable; C, constant.

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Fig. 1. Partial trypsin-Giemsa banded metaphase from hybrid 513AC3B10. The arrow indicates the 14q+ chromosome.

**In Situ Hybridization.** Chromosomal in situ hybridization was performed as described (21). The probe was pB0.9, a genomic clone containing a 0.9-kb insert, labeled with [3H]dTTP (NEN) by nick translation.

**Transverse Alternating Field Electrophoresis.** High-molecular weight DNA from various cell lines was prepared, digested, and electrophoresed using a transverse alternating field apparatus as described (22). The pulses used were 4 sec for the first 30 min and 45 sec for the following 16 hr. After electrophoresis the gel was stained in ethidium bromide (2 μg/ml) for 30 min, destained in H2O for 30 min, photographed, depurinized for 15 min in 0.1 M HCl, and transferred to nitrocellulose (0.2 μm) (Schleicher & Schuell) or Zetaprobe membranes (Bio-Rad), then baked for 2 hr at 80°C, and hybridized using described procedures (19).

**RESULTS**

**Molecular Cloning of the Two Rearranged Ji Alleles.** Rearrangements of the TCR α/β locus were examined using several genomic probes derived from a region encompassing 120 kbp of the locus for TCR-α and TCR-β (23) in DNA from the somatic cell hybrid, 513AC3B10, carrying the 14q+ chromosome (14pter→14q32::14q11→14qter) from the AT patient. No signal was detected using a probe that encompasses the Jβ region, pMI19 (23) (data not shown), indicating deletion of both Jβ alleles on the 14q+ chromosome. Use of pMI11, which is ≈58 kbp 5' to Ca (Fig. 1) and 3' to Cβ, revealed a 9-kbp BamHI restriction fragment in addition to the 13-kbp germ-line BamHI fragment (Fig. 3A). A second rearranged fragment (Fig. 3B), but no germ-line band, was detected using pMI12B3.2 (see Fig. 2), which is 5 kbp 5' to pMI11. To determine whether these non-germ-line fragments represented functional rearrangements of the TCR and/or the junction point between 14q11 and 14q32, a genomic library constructed from the hybrid 513AC3B10 was screened with nick-translated probes pMI11 and pMI12B3.2. Of ≈500,000 plaques, 6 positive clones were identified in the second screening and hybridized individually to each probe. Because the two rearrangements were not >5 kb apart (see Fig. 2) and no other human chromosome 14 sequences were present in the hybrid library, two classes of clones were delineated: (i) three clones positive for hybridization with both pMI11 and pMI12B3.5, and (ii) three clones positive with pMI11 only. Restriction analysis of two representative clones, B and H (see Fig. 2), confirmed that we had cloned both rearrangements.

**Regional Chromosomal Origin of the Two Classes of Rearranged Clones.** To determine which of the two clones represented the junction of the chromosomal translocation, somatic cell hybrid 588-AA3 DNA, which contains an 8q+ chromosome (8pter→8q24::14q11→14qter) derived from the human leukemic SKW3 cell line, the breakpoint of which has been well characterized (17), was used to map the 5' end of the clones to a region either proximal or distal to the Jβ locus. This hybrid contains sequences telomeric to the Jβ R region, which is situated 30 kb 5' to the Ca region (23). The probe pSE3.5 derived from clone H, 5' to the breakpoint (see Fig. 2), did not hybridize with 588-AA3 DNA but did segregate concordantly with chromosome 14 in other hybrid DNA (data not shown). Thus, the clone H sequences presumably derive from a region centromeric to the Ca locus. By contrast, a probe 5' to the breakpoint of clone B, pB0.9 (see Fig. 2), did hybridize with the DNA from the 588-AA3 hybrid and with DNA of other hybrids carrying chromosome 14 (see Fig. 4); thus probe pB0.9 is telomeric to the Jβ R region. Probe pB0.9 sequences are present in DNA from the 5263C17 hybrid, which retains a 14q+(14pter→14q32::Xq13→Xqter) in which...

Fig. 2. Restriction map of clones derived from germ-line sequences on 14q11, 14q32, and from the two rearranged alleles of the 513AC3B10 DNA. The two probes pMI11 and pMI12B2.3 used to clone the two rearranged clones B and H are indicated by a straight line. The probes pB0.9 (shaded bar) and pSE3.5 (solid bar) used for mapping are also shown. Probe pB0.9 was also used to clone sequences deriving from the germ-line 14q32 (top shaded line). The arrows on clones B and H indicate the diverging points from the germ-line sequences of 14q11. E, EcoRI; B, BamHI; H, HindIII; and X, XhoI.
the chromosome 14 translocation point is proximal to the D14S1 locus (13). Thus, the locus detected by probe pB0.9 is centromeric to the polymorphic D14S1 locus, which maps to 14q32.2 (24), and to the immunoglobulin heavy chain locus, which maps to 14q32.3, and telomeric to the Ja R segment. These data suggested that pB0.9 represents sequences derived from a region between 14q11 (telomeric to the Ja R loci) and 14q32.2.

Chromosomal Localization of pB0.9 by in Situ Hybridization. The precise assignment of pB0.9 was established by in situ hybridization. Grains were scored for their localization after autoradiography. The distribution of the grains on metaphases was assessed. As shown in Fig. 4, significant labeling was found over the distal portion of the long arm of chromosome 14, with a strong peak evident at 14q32.1. Of the 75 cells examined, 26 (35%) exhibited label on one or both chromosomes 14. About 16% of all grains were located on the long arm of chromosome 14, with most grains at 14q32.1. The long arm of chromosome 14 represents 3% of the haploid human genome. Our finding that 16% of the hybridization with the pB0.9 probe localized to this region of chromosome 14 is highly significant (P < 0.005). Thus, pB0.9 probe was localized to region q32.1 of human chromosome 14.

Sequences of the Two Rearranged Clones. The nucleotide sequences spanning the two rearrangements (Fig. 5) were determined. The nucleotide sequence corresponding to the BamHI/EcoRI fragment from clone H indicated a functional (variable–joining) V–J joining between the Ja U segment and a new V family member. The identification of this sequence as the Vα region was based on: (i) the presence of a long open reading frame throughout the entire sequence with a consensus motif Ala Xaa Try Xaa Cys Ala (A*V*CA) typical of the Vα region at the 3' boundary (25) (Fig. 5A) and (ii) 60–70% homology with the Vα region of the TCRα chain. This is a search of the National Biomedical Research Foundation data bank. The sequence of the other (translocated) allele was obtained from subcloning a 3.2-kb BamHI restriction fragment from clone B (Fig. 5B). To determine the breakpoint position in the chromosomal translocation, the corresponding normal 14q11 and 14q32.1 loci were cloned (see Fig. 2, top line) and sequenced (Fig. 5B). At the 14q11 site, the breakpoint occurs in a sequence lying between Ca and the Ja U segment. Comparison of this sequence with the published sequences of the Ja gene segments (25) revealed a good alignment with the consensus sequence for Ja; however, the sequence indicated a stop codon, TGA, 3' to the Phe–Gly consensus (data not shown). A heptamer–nonamer motif was also found and is indicated in Fig. 5B. A perfect donor splice could not be identified at the 3' boundary of the sequences. Taken together, these data indicate that the translocation probably involves a Ja segment, with the translocation breakpoint in the vicinity of the heptamer–nonamer 5' to this Ja. No N region was observed in the 14q14 clone. The breakpoint on 14q11 occurred 5' of the putative heptamer, indicating that a possible mistake during physiological V–J joining on 14q11 had occurred.

Pulsed Field Electrophoretic Fractionation and Analysis of High-Molecular Weight DNA Fragments. We previously reported the molecular cloning of t(7;14)(q35;q32) translocation breakpoint from a T-cell acute lymphocytic leukemia of an
We have detected and cloned two rearrangements in the TCRα locus from T lymphocytes exhibiting a t(14;14)-
(q11;q32) translocation; the lymphocytes were from an AT
patient who later developed T-cell chronic lymphocytic
leukemia. These two rearrangements represent a functional
V-J joining at 58 kb 5' to Ca and the translocation break-
point at 65 kb 5' to Ca, respectively. Functional V-J joining
on the nontranslocated allele was also reported in the case of
a chromosomal inversion [inv(14)(q11;q32)] in another pa-
tient with AT and T-cell leukemia (8). Thus, with both
patients, the capacity to properly assemble antigen receptor
genes remained intact. It is, however, possible that the
presence of a functional allele in the clonal malignant popu-
lation is required for the cell to pursue a differentiation
program in light of the fact that B and T malignant cells
 carrying a chromosomal translocation also usually produce
immunoglobulins or T-cell receptors.

The t(14;14) chromosomal breakpoint characterized here
was the result of a recombination event between a region
identified as a putative Jo on 14q11 and a previously unde-
scribed locus on 14q32.1. This chromosome translocation,
similar to the others involving chromosome 14q11 in T-cell
neoplasms (17), occurred in proximity of a heptamer on
14q11, in front of a pseudo Jo segment, suggesting that
breakage occurred during an attempt at V-J joining by
the lymphocytes of the AT patient. Chromosomal transloca-
tions and other aberrant recombination events in AT patients
frequently involve the 14q11 region, a highly active region
during T-cell differentiation, as V-J joining proceeds due to
the presence of two T-cell receptor loci, α/β, on 14q11, and
to the large number of Jo segments (>40) and two Jo
segments spanning over 100 kb. The sheer size of this 100-kb
region might contribute to errors during T-cell receptor gene
rearrangement, leading to translocations. The locus at
14q32.1 designated TCL1 is frequently involved in chromo-
some translocations in AT-associated leukemias (3, 7)
and non-AT leukemias (6). The TCL1 locus is ~10,000 kb
centeromic to and distinct from the 14q32.3 subband
where the immunoglobulin heavy chain locus maps. To date,
other chromosomal breakpoints have been localized to the
14q32.1 subband by molecular cloning (3, 6, 7) and anoth-
ern two through cytogenetic analysis (26). Breakpoints observed
in this region are not clearly clustered, a situation that resem-
bles that seen in endemic Burkitt lymphomas with the t(8;14)
translocation and in chronic myelogenous leukemia with the
t(9;22) translocation. Our transverse alternating field elec-
rophoresis data suggest comigration of probes pE/S and
Fig. 7. Model of T-cell leukemogenesis in T cells from patients with AT.

pB0.9, on a large SfI fragment, indicating that these two breakpoints may begin to define a breakpoint cluster if they can be linked by chromosome walking.

The patient presented in this study reportedly carried a clonal expansion of T cells with the t(14;14)(q11;q32) translocation 10 yr before overt leukemia. The same clone appeared to retain the capacity to differentiate along distinct maturation pathways of T helper and suppressor cells (12). In this light, the occurrence of lymphoid malignancy in AT patients does not seem unexpected. From previous reports (1, 2, 11, 26, 27), and the data presented here, the sequence of events illustrated in Fig. 7 in the development of T-cell leukemia associated with AT seems possible. Chromosomal translocations are nonrandom events in these patients and normally involve 14q11 (TCRa/β), 7q35 (TCRB), 7p14 (TCRγ), and 14q32.1 (TCL1). Although reciprocal translocations involving 14q11, 7q35, and 7p14 are not known to result in malignant transformation, the translocations involving 14q32.1 are associated with a proliferative advantage (Fig. 7) that may result in clonal expansion without clinical manifestation of leukemia. Thus the 10-year delay in appearance of overt leukemia in the present patient may reflect the requirement for a second genetic event to determine a malignant phenotype. This notion is consistent with the observations of Sparkes et al. (2), Taylor and Butterworth (11), and Levitt et al. (28), who reported additional chromosomal abnormalities in the malignant clone as compared with the nonmalignant clone and that the type of leukemia can differ from one patient to another, even in the presence of similar cytogenetic alterations. To elucidate the mechanisms involved in genesis of these T-cell malignancies the identification of sequences in the 14q32.1 region that may be responsible for initial clonal expansion is crucial.

Interestingly, other alterations, such as the inversion with duplication of a region 25 kb 5' to Cμ of the immunoglobulin heavy chain locus, were previously reported in the patient studied here (13). We detected a similar inversion with duplication on the 14q+ of another T-cell leukemia in an AT patient which had a t(7;14)(q35;q32) translocation (3). Whether this alteration has any significance for the leukemogenic process remains unclear, but this alteration does appear to occur nonrandomly in the cells of AT patients.

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