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A B-CELL LINE HAVING CHROMOSOME 14 ABERRATION AT BREAK BAND q11 DERIVED FROM AN ADULT T-CELL LEUKEMIA PATIENT

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A B-cell line having translocations of chromosome 14 at break band q11 (the assigned locus of the α-chain gene of the T-cell antigen receptor) and chromosome 3 at break band p25 (the assigned locus of the c-*raf*-1 oncogene) was established from peripheral blood leukocytes of an adult T-cell leukemia (ATL) patient. The same chromosome 14 aberration at break band q11 and chromosome 3 aberration at break band p25 were also found in fresh T-cell leukemia cells. The B-cell line is surface immunoglobulin (sIg)$^+$, immunoglobulin gene rearrangement$^+$, ATL-specific antigen (ATLA)$^+$, HTLV-1 proviral genome$^+$, Epstein-Barr virus (EBV)-associated nuclear antigen (EBNA)$^+$ and the EBV DNA genome$^+$. The fresh T-leukemic cells were T-cell receptor gene rearrangement$^+$, the HTLV-1 proviral genome$^+$ and EBV DNA genome$^-$.

Key words: ATL — B-cell line — HTLV-1 — EBNA — Chromosome 14

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Adult T-cell leukemia/lymphoma (ATL, ATLL) is a newly recognized malignant disease of T-lymphocytes which occurs in Japan.[1] Leukemic cells of ATL have been observed to have an inducer/helper T-cell antigen phenotype (OKT4 and/or Leu3 positive). The human T-cell leukemia/lymphoma virus type 1 (HTLV-1) is an identified retrovirus which has been associated with ATL.[2, 3] Specific abnormalities in chromosome 14 at break band q11 (the assigned locus of the α-chain gene of the T-cell antigen receptor) have been reported in some human T-cell malignancies involving ATL.[4–11] We have established a B-cell line having translocations of chromosome 14 at break band q11 and chromosome 3 at break band p25 (the assigned locus of the c-*raf*-1 oncogene).

The patient, a 56-year-old man, was admitted to the city hospital of Uwajima in July, 1985, with expectoration and coughing; upon examination, he showed generalized hepatomegaly. Relevant data from laboratory investigations of the patient are shown in Table I. His blood leukocyte count was 67,000 per mm^3, with 80% leukemic cells, which had indented or lobulated nuclei. The patient was diagnosed as typical ATL. The surface markers of fresh leukemia cells were OKT3$^+$, OKT4$^+$, OKT8$^-$ and OKT11$^+$ and he had antibodies against EBV and HTLV-1. A chromosome analysis of fresh T-leukemic cells from the patient revealed chromosome 14 aberrations with a break at band q11. The detailed bimodal karyotypes were 46,XY,t(3;14)(p25;q11) and 47,XY,−13−18,+21,t(3;14)(p25;q11),del(5)(q13q22),+der(13)t(3;13)(p13;p11),+der(18)t(18;?)(q23;?)(case 5 in our previous report, 1987).[11]

The patient's peripheral blood mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation and cultured at 5 $\times 10^6$ cells per ml in 60-mm Petri dishes with RPMI 1640 medium supplemented with 10% human cord serum, 10% fetal calf serum and antibiotics. The cultures were then incubated at 37° in a humidified 7.5% CO_2 atmosphere

Table I. Characteristics of Fresh Leukemic Cells and the Cell Line

	Fresh leukemic cells	Cell line
OKT3 and/or Leu 4	87.0%	0%
OKT4 and/or Leu 3a	90.0%	0%
OKT8 and/or Leu 2a	1.0%	0%
OKT11 and/or Leu 5	95.2%	0%
OKIa1 and/or HLA-DR	64.6%	81.0%
Tac		66.1%
B1	0%	74.9%
B4	0%	89.6%
IgA		<1.0%
IgM		42.0%
IgG		<1.0%
T-cell receptor gene rearrangement	+	germ line
Human immunoglobulin heavy chain rearrangement		+
EBNA	−	>80.0%
ATLA		1–2%
EBV DNA genome	−	+
HTLV-1 proviral genome	+	+
Chromosome karyotype	46,XY,t(3;14)(p25;q11)*/ 47,XY,−13,−18,+21,t(3;14)(p25;q11),del(5)(q13q22),+der(13)t(3;13)(p13;p11),+der(18)t(18;?)(q23;?)	46,XY,t(3;14)(p25;q11)

*This karyotype in fresh leukemic cells has been reported previously.[6]
+, positive; −, negative.

and fed twice a week. After about one month of culturing, the cells began to proliferate and have since been subcultured about every 4–5 days. The cell line grows forming clusters in suspension with a doubling time of about 34 hr. Its cell surface characteristics are listed in Table I. It is a B lymphocyte line expressing immunoglobulin, Ia molecules, HLA-DR, the receptor for T cell growth factor (TCGF), and the B-cell differentiation markers B1 and B4. The B-cell line does not express any of the T-cell markers (OKT3, OKT4 and OKT11). Carbon tetrachloride- or acetone-fixed cells were examined for EBNA and ATLA by indirect immunofluorescence as described previously.[12] To detect EBNA, standard sera obtained from healthy persons were used. For ATLA staining, we used mouse monoclonal antibodies (GIN-14) raised against p19 as described before.[12] The cell line was positive for EBNA and ATLA. Chromosome analysis revealed a chromosome 14 abnormality with a break at band q11, as was also seen in the fresh T-cell leukemic cells. The karyotype was 46,XY,t(3;14)(p25;q11) in all of the examined 100 metaphases (Fig. 1). The break band in chromosome 3 was in band p25, a region reported to include the locus of the c-*raf*-1 oncogene.

The clonal T-cell or B-cell nature of the fresh leukemic cells and the cell line was confirmed by analysis of the configuration of the T-cell receptor β-chain gene or the human immunoglobulin gene. High-molecular-weight DNA was extracted from the fresh leukemic cells and the cell line and digested with restriction endonuclease, *Eco* RI or *Bam* HI. The DNA fragments obtained were subjected to 0.7% agarose gel electrophoresis and transferred to a Hybond-N filter by the method of Southern. Filters were hybridized with a ^{32}P-labeled human T-cell receptor β-chain probe ($C\beta_1$) or the J region of a human immunoglobulin heavy chain probe (JH). Figure 2 (A, B) shows the results of probing of the fresh leukemic cells and the cell line DNA with $C\beta_1$ probe and JH probe, respectively. The fresh leukemic cells (Fig. 2A, lane 1)

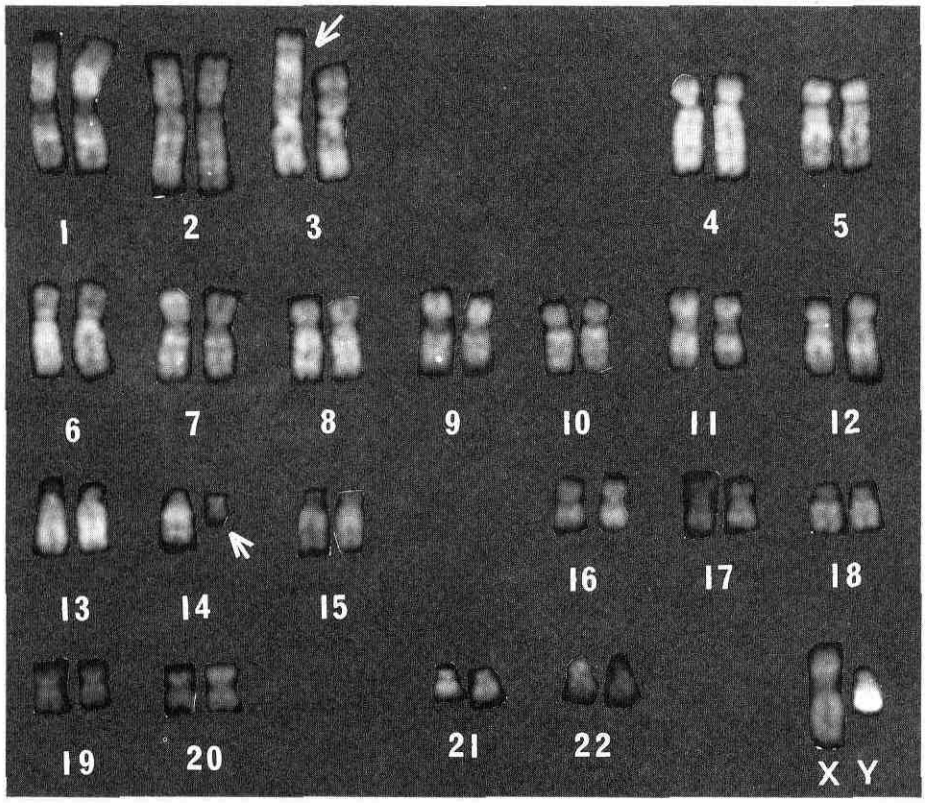

Fig. 1. Karyotype of leukemic cell from the B-cell line with t(3;14)(p25;q11) translocation. The break point on chromosome 14 is at band q11 and that on chromosome 3 is at band p25. Arrows indicate t(3;14)(p25;q11).

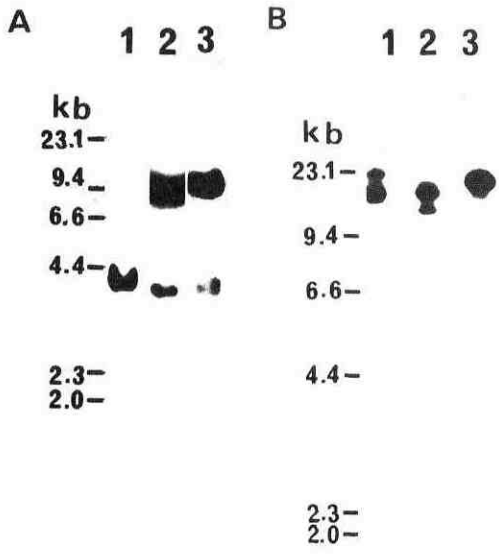

Fig. 2. (A) β-Chain gene rearrangements in fresh leukemic cells and the B-cell line; lane 1 shows a complete rearrangement of the T-cell receptor gene (fresh cells); 2 shows a complete germ line configuration (B-cell line); 3 shows a negative control (HL-60 cell line) (digested by *Eco* RI). (B) Southern blot hybridization using the J region of the human immunoglobulin heavy chain. Lane 1 shows an immunoglobulin gene rearrangement (B-cell line); 2 shows a positive control (BATL-7)[12]; 3 shows a negative control (HL-60 cell line) (digested by *Bam* HI).

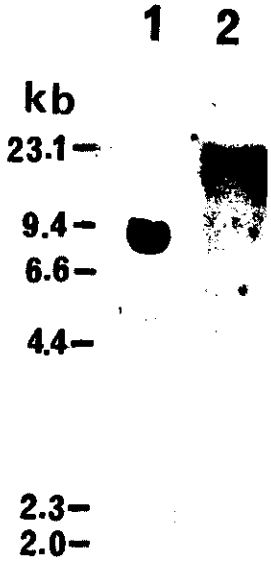

Fig. 3. Detection of proviral DNA sequences of HTLV-1 in fresh leukemic cells and the cell line. Cellular DNA (10 μg) was cleaved with *Eco* RI and electrophoresed on 0.8% agarose gel. The gels were blotted onto nitrocellulose and hybridized. The probe used was pMT-2, which covers the whole genome of the HTLV-1 virus. Lane 1, fresh T-cell leukemia cells; 2, B-cell line (digested by *Eco* RI).

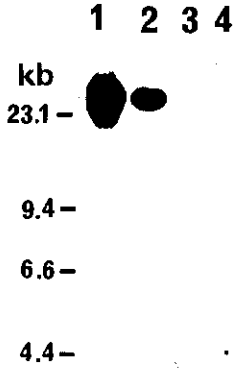

Fig. 4. Identification of EBV DNA in the B-cell line by Southern blot hybridization. Cellular DNA (10 μg) was cleaved with *Eco* RI or *Bam* HI and electrophoresed on 0.7% agarose gel. The gels were blotted onto nitrocellulose and hybridized. The probe used consisted of *Bam* HI-W fragments of EBV DNA (B95-8 strain).[18] Lane 1, B-cell line; 2, BATL-7 (positive control)[12]; 3, fresh T-cell leukemia cells; 4, HL-60 (negative control) (digested by *Eco* RI).

exhibited complete rearrangement of the T-cell receptor gene and demonstrated a mature differentiated T-cell character. The cell line (Fig. 2A, lane 2) exhibited a complete germ line configuration. The cell line showed complete rearrangement with the JH probe (Fig. 2B, lane 1).

In order to characterize the integration sites of the HTLV-1 and EBV viruses in the fresh leukemic T-cells and the cell line, we performed Southern analysis on DNA isolated from these cells. In brief, cellular DNA was digested with *Eco* RI or *Bam* HI. The DNAs separated by agarose gel electrophoresis were blotted onto a nitrocellulose filter and hybridized with cloned HTLV-1 ^{32}P-labeled probe or EBV DNA ^{32}P-labeled probe. The presence of only one band of HTLV-1 proviral DNA in the fresh leukemic cells indicates that the leukemic cells were monoclonal (Fig. 3, lane 1). The B-cell line had multiple integrated proviral copies (Fig. 3, lane 2). A difference in the integration sites of HTLV-1 was found between T-cells and B-cells, indicating that the chromosome 14 aberration may not be directly associated with the HTLV-1 integration sites of leukemic cells. EBV DNA was detected in the cell line (Fig. 4, lane 1), but not in the fresh leukemic cells (Fig. 4, lane 3). This was probably because the population of EBV DNA B-cell was small in the fresh leukemic cells.

To demonstrate the immortalization of either T or B lymphocytes through HTLV-1 transformation by co-cultivation with the B-cell line, after irradiation (10,000 R), we co-cultured the B-cell line with anti-HTLV-1 negative lymphocytes from a healthy person. The transformed cells had ATLA-positive (>90%) and OKT3, OKT4-positive surface markers (data not shown). It has been reported that HTLV-1-infected B-lymphocytes have been isolated from the peripheral blood of ATL patients.[12-14] These results suggest that the HTLV-1 virus may be associated with a broader range of host cells.[15]

Our results suggest that the chromosome 14 aberration at break band q11 is associated not only with T-cell malignancies but also with B-cell malignancies. The tumor development may be associated with c-*raf*-1 oncogene deregulation by chromosome translocation involving juxtaposition of the oncogene and the

α-chain gene of the T-cell antigen receptor *in vivo*. However, it is also possible that c-*raf*-1 oncogene was deregulated by the t(3;14) translocation through juxtaposition of this gene not with the T-cell antigen receptor α-chain gene, but with an unknown gene located also in band 14q11, and that rearrangement of the T-cell receptor α-chain gene occurred afterward only in the T-cell. On the other hand, B-cells having t(3;14) translocation may not be deregulated for the c-*raf*-1 oncogene *in vivo*. Further study of the B-cell line should contribute to a better understanding of the mechanism of the pathogenesis of ATL.

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