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DNA segment containing $C_{\beta 1}$, a gene for the constant region of the β chain of the T-cell antigen receptor, was inserted into chromosome 6 in cells from one patient with human T-cell leukemia

(DNA rearrangement/reciprocal joint-containing segment/tumorigenesis)

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ABSTRACT **DNA rearrangements that occurred in the vicinity of T-cell antigen receptor β-chain gene clusters residing on chromosome 7 were examined in human T-cell acute lymphoblastic leukemia cells. In one patient, it was observed that, for the T-cell receptor β-chain genes, a $D_{\beta 1}$-$J_{\beta 2.3}$ (where D is diversity and J is joining) junction was found on one chromosome, while the other chromosome kept the germ-line configuration. If this D_β-J_β junction was formed by the customary deletion mechanism, the $C_{\beta 1}$ gene (where C is constant) located between the $D_{\beta 1}$ and $J_{\beta 2.3}$ loci should have disappeared from this chromosome. The $C_{\beta 1}$ gene indeed was absent from the rearranged chromosome 7, but it was found on chromosome 6 as an inserted segment. The implications of the observations are discussed.**

Various chromosome translocations have frequently been observed in human leukemic cells (1). For example, chronic myelocytic leukemia is characterized by the presence of Philadelphia chromosome, which is the result most often of reciprocal translocation between chromosome 22 and 9 (2). As a result, *ABL* oncogene, which is the human cellular counterpart of the transforming gene of Abelson murine leukemia virus (v-*abl*) normally located on chromosome 9, is translocated to the Philadelphia chromosome (3). The break point on chromosome 22 is restricted to the break-point cluster region (*BCR*). Similarly, the break on chromosome 9 also occurs at the fixed point ≈14 kilobases (kb) on the 5′ side of the *ABL* gene (4). In Burkitt lymphomas, the following three kinds of translocations have been identified: t(8;14), t(8;22), and t(2;8) (5). These reciprocal translocations bring the *MYC* oncogene located on chromosome 8 to the vicinity of the immunoglobulin heavy (H)-, λ-, and κ-chain gene clusters residing on chromosomes 14, 22, and 2, respectively (6). There have been reports of other translocations; e.g., t(11;14) in chronic B-cell lymphomas and leukemias (7) and t(14;18) in acute pre-B-cell leukemia (8). These translocations led to the identification of additional putative oncogenes: *BCL1* on chromosome 11 and *BCL2* on chromosome 18 (9, 10).

The T-cell antigen receptor is rather similar to the immunoglobulins (11). It consists of a heterodimer made up of α and β chains (12). N-terminal regions of both chains are variable and form a dimeric antigen-combining pocket. The variable (V) region of β chain is encoded by the three split genes V_β, D_β, and J_β (where D stands for diversity and J stands for joining) (13). Successive DNA rearrangements first between D_β and J_β, and then between V_β and D_β form a complete active *V* gene. In this study, we have examined the DNA rearrangements in the vicinity of T-cell antigen receptor β-chain gene cluster in cells from one patient with human T-cell acute lymphoblastic leukemia (T-ALL). We found an unusual rearrangement pattern, which suggests a pathway to induce nonregulated expression of oncogenes in T lymphocytes.

MATERIALS AND METHODS

The cDNA clone of the mouse T-cell antigen receptor β-chain gene 86T5 was donated by M. Davis (11). The Maniatis human genomic library (14) was screened using 86T5 as a probe. Three overlapping clones (λHTβ-1, λHTβ-2, and λHTβ-3) covered the region of human T-cell receptor D_β, J_β, and C_β gene loci (where C stands for constant). The patient was a 23-year-old male with leukocytosis and a mediastinal mass, and was diagnosed as having acute lymphoblastic leukemia. Tumor cells were isolated from the patient's peripheral blood, before any therapy, by centrifugation on a conventional Ficoll/Hypaque density gradient. The purified cell suspension contained >95% tumor cells. Cell surface marker analyses showed that sheep erythrocyte receptors, CD2 and CD5, were expressed, but no others (e.g., CD1, CD3, CD4, or CD8) on this tumor. High molecular weight DNA was prepared by the published method (15). Southern hybridization was carried out by the same method as described (16). Rearranged clones were isolated by the ordinary cloning procedure as described by Sakano *et al.* (17). The nucleotide sequence was determined by the Maxam–Gilbert method (18).

RESULTS

Unusual Rearrangements of T-Cell Antigen Receptor β-Chain Gene Loci in Cells from One T-ALL Patient. Fig. 1 shows the restriction map of human C_β gene loci (20) and the probes used in this study. Three kinds of probes were prepared. The mouse $C_{\beta 1}$ probe can detect both the $C_{\beta 1}$ and $C_{\beta 2}$ coding regions of man as equally intense bands in the Southern blots. It detects the 2.2 kb band for $C_{\beta 1}$ and an 8.8-kb band for $C_{\beta 2}$ in *Xba* I digests of germ-line DNA as well as the 9.6-kb band for $C_{\beta 1}$ and the 3.6-kb band for $C_{\beta 2}$ in *Eco*RI digests. The rearrangements in $J_{\beta 1}$ gene cluster and those in $J_{\beta 2}$ gene cluster can be detected in *Eco*RI digests and *Xba* I digests, respectively. Rearrangements of D_β-J_β or V_β-D_β can be identified indirectly by using $D_{\beta 1}$ and $J_{\beta 2}$ probes (Fig. 1). $D_{\beta 1}$ and $J_{\beta 2}$ probes detect distinct fragments.

Unusual patterns were observed in the Southern blots from the T-ALL patient we studied (Fig. 2). The probes detected

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Abbreviations: T-ALL, T-cell acute lymphoblastic leukemia; V, variable; D, diversity; J, joining; C, constant; β, β chain; H, heavy; L, light.

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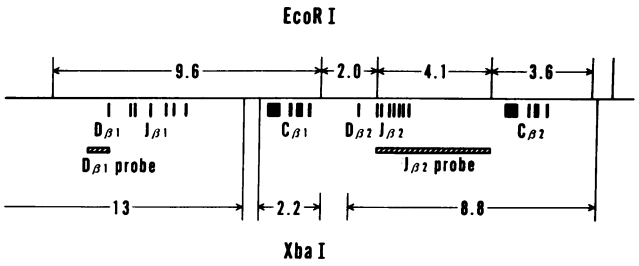

FIG. 1. Restriction map of human T-cell receptor β-chain gene loci. Three overlapping clones (λHTβ-1, λHTβ-2, and λHTβ-3) covered the region of human T-cell receptor β-chain gene loci. The restriction map is essentially the same as published (19). The $D_{\beta 1}$ probe corresponds to the 0.9-kb *Kpn* I–*Hin*dIII fragment. The $J_{\beta 2}$ probe corresponds to the 4.1-kb *Eco*RI fragment.

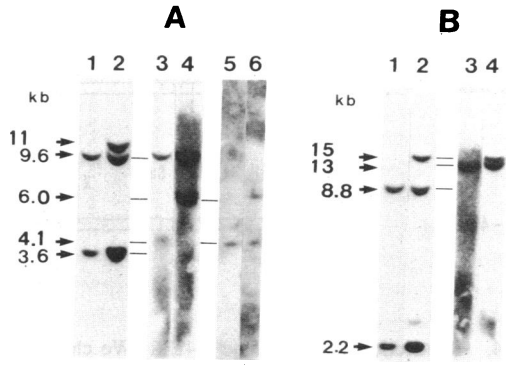

FIG. 2. Southern blot analysis of DNA from T-ALL. DNA was digested with *Eco*RI or *Xba* I and subjected to 0.8% agarose gel electrophoresis. (*A*) *Eco*RI digests. (*B*) *Xba* I digests. Germ-line DNA (lanes 1, 3, and 5). T-ALL DNA (lanes 2, 4, and 6). C_β probe (lanes 1 and 2). $D_{\beta 1}$ probe (lanes 3 and 4). $J_{\beta 2}$ probe (lanes 5 and 6).

one rearranged band in addition to the germ-line bands in all the *Eco*RI and *Xba* I digests. The above suggested the coexistence of one chromosome 7 that underwent V_β, D_β, and J_β rearrangement and the other chromosome 7 that remained intact. The possibility of trisomy of chromosome 7 was excluded by karyotype analysis (data not shown). In the *Xba* I digests, a 15-kb band detected by the $C_{\beta 1}$ probe was also detected by the $D_{\beta 1}$ probe. In the *Eco*RI digests, the $J_{\beta 2}$ probe and the $D_{\beta 1}$ probe detected a band at 6 kb. This indicates that $D_{\beta 1}$-$J_{\beta 2}$, rather than $D_{\beta 1}$-$J_{\beta 1}$ rearrangement, occurred in this tumor. Since $C_{\beta 1}$ normally resides between $D_{\beta 1}$ and $J_{\beta 2}$, this rearrangement should have deleted $C_{\beta 1}$ gene (20). The C_β probe, however, detected three bands at 11 kb, 9.6 kb, and 3.6 kb in *Eco*RI digests. The 9.6-kb and 3.6-kb bands corresponded to germ-line $C_{\beta 1}$ and $C_{\beta 2}$ genes, respectively, retained by the intact chromosome 7. The 11-kb band might have contained the $C_{\beta 2}$ gene involved in the rearrangement. However, $J_{\beta 2}$ probe didn't detect this 11-kb band, and if this band contained $C_{\beta 2}$, there should have been two tandem *Eco*RI sites downstream of the $C_{\beta 2}$ gene (Fig. 1). Accordingly, this 11-kb band detected by C_β probe in *Eco*RI digests remained a puzzle.

$D_{\beta 1}$-$J_{\beta 2.3}$ Rearrangement on One Chromosome and Germ-Line Configuration on the Other Chromosome. The 6.0-kb fragment detected by $J_{\beta 2}$ probe in *Eco*RI digests was cloned. Restriction map analysis of the clone indicated the presence of the *Nco* I site (20) located 10 nucleotide upstream of $D_{\beta 1}$; lack of the *Hin*dIII site 150 nucleotides downstream of $D_{\beta 1}$; and the absence also of the *Sac* I site (20) located between $J_{\beta 2.2}$ and $J_{\beta 2.3}$. However, the *Sac* I site between $J_{\beta 2.6}$ and $J_{\beta 2.7}$ remained. The DNA sequence around the rearranged point showed the DNA rearrangement between $D_{\beta 1}$ and $J_{\beta 2.3}$, and the presence of a 5-nucleotide insertion known as the N segment (21) at the boundary (Fig. 3). It thus appeared that, in this tumor cell clone, $D_{\beta 1}$-$J_{\beta 2.3}$ joining had occurred on one chromosome whereas the other chromosome kept the germ-line configuration.

Discarded $C_{\beta 1}$ Was Inserted into Chromosome 6. We cloned the 11-kb band detected by C_β probe in the *Eco*RI digests. Fig. 4 shows the restriction map of the clone. Although the boundary between the C_β gene-containing fragment and the unknown fragment has not been determined precisely, the *Sca* I site (20) located between $J_{\beta 1.2}$ and $J_{\beta 1.3}$ was retained, whereas the *Hin*dIII site (20) located at the inside of the $J_{\beta 1.1}$ coding region was not. Therefore, one break point must have been in the vicinity of $J_{\beta 1.2}$. To carry out chromosome assignment of the unknown fragment connected to the C_β gene-derived fragment, 13 DNAs from mouse–human somatic cell hybrids that have selectively retained small numbers of human chromosomes were used in Southern blots. Probe I shown in Fig. 4 identified a band at 9.6 kb in *Eco*RI-digested human DNA and four faint bands at 11, 9.0, 6.5, and 5.0 kb in *Eco*RI-digested mouse DNA. The DNA from 3A6 and A/B7-5 cells gave a signal at 9.6 kb, but the DNAs from the other cells gave only the signals derived from mouse genome. From the karyotype analyses data for the hybrid cells listed in Table 1, we assigned this unknown fragment to human chromosome 6.

DISCUSSION

The mechanisms of V_L-J_L as well as V_H-D_H-J_H DNA rearrangements have been extensively examined in the case of immunoglobulin gene clusters (where L stands for light and H stands for heavy). In the vicinities of germ-line *V*, *D*, and *J* genes are the characteristic heptamer CACAGTG or CACTGTG and nonamer ACAAAAACC or GGTTTTTGT (22). These oligomers are separated by regular spacers. In the case of the relevant combinations V_λ-J_λ, V_κ-J_κ, V_H-D_H, and D_H-J_H, one partner is endowed with a 12-nucleotide spacer, whereas the other partner is equipped with a 23-nucleotide spacer (23). The putative recombinase that mediates such DNA rearrangements is believed to recognize both the sequences and the spacer length. In T-cell receptor gene systems, the presence of the same set of signal sequences has been observed (13). It thus appears that both immunoglobulin and T-cell receptor genes utilize the same recombination machinery (13). Such DNA rearrangements usually delete long stretches of DNA (22). When one *D* gene and one *J* gene

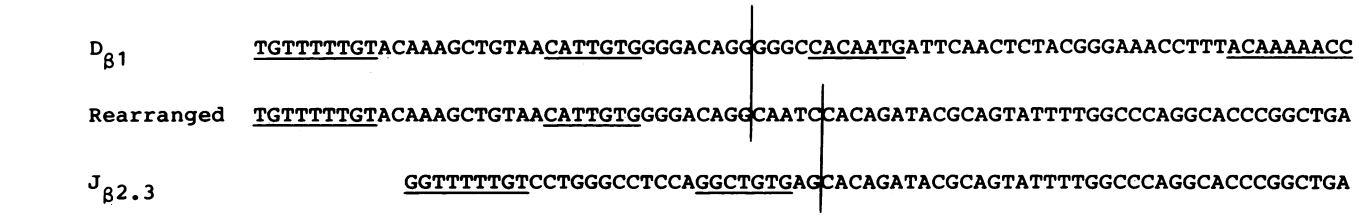

FIG. 3. Nucleotide sequence of the rearranged region. Nucleotide sequences around $D_{\beta 1}$ and $J_{\beta 2.3}$ germ-line genes (19) are shown for the comparison. The signal heptamers and nonamers are underlined. Breaking points are shown by vertical lines.

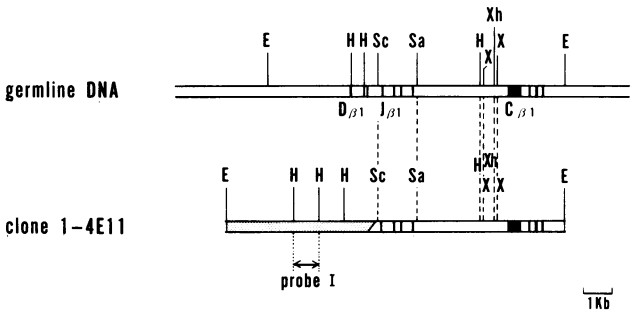

FIG. 4. Restriction map of clone 1-4E11. We cloned the 11-kb band detected by C_β probe in the *Eco*RI digests. The clone is named 1-4E11. Approximately 6 kb of the fragment is derived from the C_β gene. The map around the germ-line $C_{\beta 1}$ gene locus is shown for the comparison. The coding region is indicated by black boxes. The breaking point is located at approximately $J_{\beta 1.2}$ locus. Probe I shown by arrows was used for chromosome identification. E, *Eco*RI; X, *Xba* I; Xh, *Xho* I; H, *Hin*dIII; Sa, *Sac* I; Sc, *Sca* I.

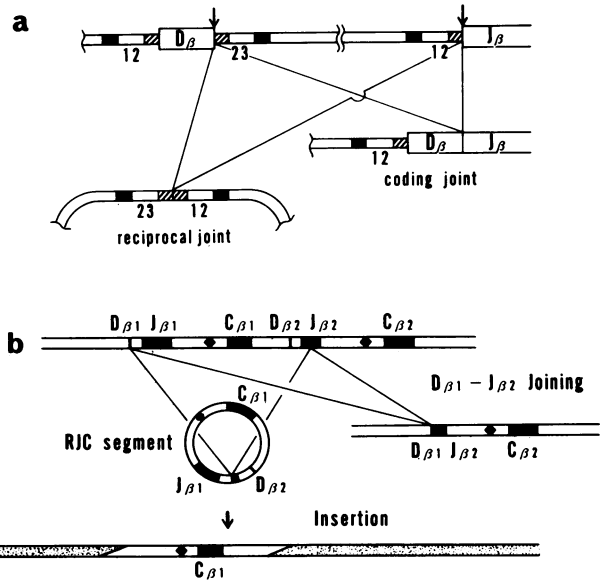

FIG. 5. (*a*) Scheme of D_β-J_β joining. (*b*) Scheme of insertion of the reciprocal joint-containing (RJC) segment into another chromosome.

join, the intervening DNA sequence is discarded from chromosomal DNA. In certain cases, where the polarity of the genes embedded in the chromosome is opposite, however, inversion occurs (24). The above indicates the following reciprocal recombination process (Fig. 5*a*): The recombinase recognizes two sets of the signal heptamer and nonamer, one of which contains a 12-nucleotide spacer whereas the other contains a 23-nucleotide spacer. An endonuclease first cuts the side of the signal heptamer. A ligase joins both heptamers, resulting in a head-to-head structure referred to as a "reciprocal joint" (25). The edges of the coding regions neighboring the heptamers are processed by an exonuclease and ligated to form a "coding joint" (25).

The organization of T-cell receptor β-chain gene cluster is shown in Fig. 5*b*. Twelve-nucleotide spacer signal sequences are present at the 5′ sides of D_β genes and J_β genes, and 23-nucleotide spacer signal sequences are at the 3′ sides of V_β genes and D_β genes (20). If DNA rearrangement occurs between $D_{\beta 1}$ and one of the $J_{\beta 2}$ genes, the DNA fragment that separates both genes takes on a circular form and, since it cannot be replicated, is normally lost from the cell. However, instead of being lost, this putative "reciprocal joint"-containing DNA fragment might be inserted into another chromosome locus by using a general recombination system as shown in Fig. 5*b*. We tentatively refer to this fragment as a "reciprocal joint-containing segment". Even if this pathway exists, it may not be detected in normal T cells because of low frequency. The presence of an enhancer-like sequence has been presumed in the 5′ direction from each C_β gene (26), as in immunoglobulin gene systems (27). If so, the reciprocal joint-containing segment may contain the enhancer-like sequence. It thus appears plausible that the insertion of the $C_{\beta 1}$-containing fragment into chromosome 6 has a potential to induce the nonregulated expression of a certain gene located in the vicinity of the insertion point. If such a gene is one of the oncogenes, the observed insertion might have contributed to the manifestation of T-cell leukemia.

Many malignant cells of neoplasias have characteristic chromosomal abnormalities that have been identified mainly by karyotype analyses (1). Although ≈10% of adult patients diagnosed as having chronic myelocytic leukemia are known to lack the Philadelphia chromosome, Morris *et al.* (28) reported that two of the five patients of this type showed the same genomic change as in Philadelphia chromosome-positive chronic myelocytic leukemia, but that the change resulted from a mechanism other than reciprocal translocations. This indicates that the frequency of chromosome defects in cancer cells should be higher than the value estimated from chromosome-banding analyses because insertions of small DNA segments into chromosomes cannot be readily identified by such analyses.

In this paper we have described a particular pathway leading to insertion of a part of the T-cell receptor gene

Table 1. Mouse–human somatic cell hybrids with selected human chromosomes

Hybrid clone	Human chromosome																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
1-4	−	−	−	+	−	−	+	+	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+	−
1-5	+	−	−	+	−	−	+	+	−	−	+	+	−	−	−	+	+	+	−	+	+	−	−	−
1-6	−	−	+	+	+	−	+	−	−	+	+	+	−	−	+	−	+	+	+	−	−	−	+	−
2-2	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	+	+	+	−	+	+	+	−
3-2	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−
3-4	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	+	+	−
7-1	−	−	+	−	−	−	−	−	−	+	−	+	+	+	−	−	+	−	−	+	+	−	−	−
1B1-24	−	−	+	−	−	−	+	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−
3A6	−	−	+	+	−	+	−	+	−	−	−	−	−	−	−	+	+	+	−	+	+	−	+	−
3B5	−	−	−	+	+	−	−	−	−	−	−	−	+	+	−	−	+	+	−	+	+	−	−	−
7-1A2	+	−	+	−	−	−	+	−	−	−	−	−	+	+	+	−	+	−	+	+	−	−	−	−
7-1D2	−	−	+	−	−	−	−	−	−	−	+	−	+	+	−	−	−	−	−	+	−	−	−	−
A/B7-5	+	+	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−

+, Present; − not present.

cluster into another chromosome. Reciprocal joint-containing segments containing the $C_{\beta 1}$ gene can also be generated by other means. One of the V_β genes was shown to be located downstream from the $C_{\beta 2}$ gene on the germ-line genome (29). However, the majority of V_β genes are believed to be encoded in a cluster upstream of the $D_{\beta 1}$ gene (30). When DNA rearrangement occurs between one of the V_β genes and $D_{\beta 2}$ gene after $D_{\beta 2}$-$J_{\beta 2}$ joining, the DNA segment that separates both genes should make a circular reciprocal joint-containing segment that could be inserted into another chromosome as well.

As described above, the reciprocal joint-containing segment was inserted into human chromosome 6 in the T-ALL cells examined in this study. On chromosome 6, *MYB* and *RAS1* have been identified (31, 32), although *KRAS1* is only a processed pseudogene (33). The character of the *MYB* gene appears to be very similar to that of the *MYC* gene. It has been reported that the *MYB* gene encodes a nuclear DNA-binding protein (34) and is expressed predominantly in hematopoietic cells (35). Expression of *MYB* has been detected in all hematopoietic lineages (36) but appears to be switched off during differentiation. Deregulation of the expression of *MYB* gene by retrovirus insertion (37) or gene amplification (38) seems to be related to tumorigenesis of hematopoietic cells. It would be of great interest to find if the observed insertion into chromosome 6 was in close proximity to the *MYB* gene. Even if not, it is still possible that chromosome 6 carries other oncogenes yet to be identified. Screening of T-cell leukemia or T-cell lymphoma by the method similar to the one presently described may lead to the identification of other unknown oncogenes.

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