THE CHROMOSOME TRANSLOCATION (11;14)(p13;q11) ASSOCIATED WITH T CELL ACUTE LEUKEMIA

Asymmetric Diversification of the Translocation Junctions

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Cytogenetic studies have identified chromosome defects that are consistently associated with particular types of tumors, and therefore may represent genetic events that promote neoplastic development (1). Little is known about the mechanisms by which such chromosome abnormalities arise. Nevertheless, chromosome defects associated with lymphoid tumors frequently feature cytogenetic breakage within the Ig or TCR genes. Moreover, breakage usually occurs adjacent to the rearranging elements of these loci, e.g., the V, D, and J gene segments that recombine during normal lymphoid development (2). This phenomenon has fueled speculation that chromosome defects involving the Ig/TCR loci arise due to aberrant activity of the same recombinase that mediates normal V-J and V-D-J rearrangement.

Gene rearrangements within the Ig/TCR loci are directed by recombination signals that flank the rearranging gene segments and presumably serve as recognition sites for the Ig/TCR recombinase (3–5). During normal Ig-TCR gene rearrangment, two reciprocal products of recombination are generated: a "coding joint," which constitutes the fusion of two gene segments of the rearranging locus; and a "signal joint," comprised of the two recombination signals that had previously flanked the rearranged gene segments. A striking feature of the recombination process is its asymmetry. Signal joints are usually formed in a conservative fashion without the loss or gain of nucleotides at the recombination junction. In contrast, coding joints are diversified as a result of both random trimming and random addition of nucleotides at the rearrangement site; the diversification of coding joints in turn contributes significantly to the generation of antibody diversity during the immune response.

The chromosome translocation t(11;14)(p13;q11) is observed in the malignant cells of 5–20% of patients with T cell acute lymphocytic leukemia (T-ALL)[1] (6–11). The translocation generates a reciprocal exchange of genetic material between chromo-

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[1] *Abbreviations used in this paper:* bcr, breakpoint cluster region; PCR, polymerase chain reaction; T-ALL, T cell acute lymphocytic leukemia.

somes 11 and 14, with cytogenetic breakpoints at chromosomal positions 11p13 and 14q11. Breakage on chromosome 14 occurs within the TCR-α/δ chain locus (12, 13), and often involves gene segments that encode the TCR-δ polypeptide (14–17). Hence, it has been proposed that t(11;14)(p13;q11) promotes tumor formation by the activation of a proto-oncogene from 11p13 upon its transposition into the TCR locus. Although the putative proto-oncogene has yet to be identified, support for this hypothesis is implied by the fact that 11p13 breakpoints from different patients are confined to a discrete 1–2-kb region, designated the 11p13 breakpoint cluster region (bcr) (14).

The t(11;14)(p13;q11) translocation generates two abnormal chromosomes, designated $11p^+$ and $14q^-$, each of which bears one of the two reciprocal translocation junctions (6). Here we describe t(11;14)(p13;q11) translocations from two patients with T-ALL. In each case, the translocation involved rearrangement between a recombination signal associated with a TCR Dδ gene segment and a pseudo recombination signal within the 11p13 breakpoint cluster region. Moreover, analysis of the reciprocal translocation junctions reveals a strict asymmetric pattern of junctional diversification typical of that observed in normal Ig/TCR gene rearrangements, and as such, provides compelling evidence implicating the Ig/TCR recombinase in chromosomal rearrangements of lymphoid tumors.

Materials and Methods

The Patients. The clinical history and specimens of patient 1114 have been described (17). Patient 2114 was a 29-yr-old male diagnosed with T-ALL in August 1988. At presentation, he had a total white cell count of 130,000/mm^3, which were mostly lymphoblast. 90% of these lymphoblasts were TdT^+, $CD2^+$, and surface Ig^-. The patient received standard T-ALL therapy and achieved a complete remission. Follow-up bone marrow examinations were performed in January and March 1989; neither showed evidence of residual leukemia. In the following months, the patient's neurological status quickly deteriorated, terminating in complete paralysis in July 1989, and death. No evidence of residual leukemia could be detected. Patient 2114 specimens analyzed in this study include aliquots of peripheral blood obtained immediately before (tumor sample 1) and 8 h after (tumor sample 2) commencement of chemotherapy in August 1988; a remission sample of peripheral blood was obtained in March 1989.

DNA Analysis and Cloning. DNA extracted from patient specimens was analyzed by Southern hybridization with radiolabeled DNA probes (18, 19). Genomic DNA libraries of Bam HI- and Xba I-digested tumor DNA from patient 2114 were constructed in phage vector λ2001 (20). These libraries were screened by the method of Benton and Davis (21), and restriction fragments of recombinant λ DNA were subcloned into plasmid and M13 phage vectors (22, 23). Nucleotide sequence analyses were performed on M13 single-stranded templates by the chain terminator method (24).

Polymerase Chain Reaction (PCR). Amplification of tumor DNA from patient 1114 was conducted by the PCR (25), with oligonucleotide primers complementary to a chromosome 14 sequence (GTATTGTGGATCCCAGCGGGTGGT) and a chromosome 11 sequence (AATGGTACCCACTTTGCAGGGTTG). Each reaction consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 1 μM each oligonucleotide primer, 125 μM each deoxynucleotide triphosphate, 1 μg tumor DNA, and 0.25 U Taq DNA polymerase (Perkin-Elmer Cetus). Reaction volumes were 100 μl under 75 μl of mineral oil. The reactions were subjected to 60 cycles of denaturation (1 min at 94°C), primer annealing (3 min at 55°C), and extension (3 min at 72°C). Temperature cycling was carried out in an automated heating/cooling block (DNA Thermal Cycler; Perkin-Elmer Cetus). The reactions were fractionated by electrophoresis on a 10% polyacrylamide gel, and the 197-bp amplification product was visualized by ethidium bromide staining. After elution from the gel, the amplification product was digested with restriction enzymes Bam HI and Asp 718, and cloned into M13 phage vectors for nucleotide sequence analysis.

Results

t(11;14)(p13;q11) Translocation Identified by Southern Hybridization. We have shown that the t(11;14)(p13;q11) translocation of a 2-yr-old boy with T-ALL (patient 1114) arose due to aberrant rearrangement between a recombination signal associated with the TCR Dδ2 gene segment and a pseudo recombination signal within the 11p13 bcr (17). Isolation of the $11p^+$ chromosome junction from this patient provided a DNA probe (2BE-2.4) that can be used to detect rearrangement of the 11p13 bcr in other patients. As shown in Fig. 1 *A*, Southern analysis with this probe revealed rearrangements in leukemic DNA of a patient (2114) for whom cytogenetic data were not available. Two rearranged Bam HI fragments were detected: a strongly hybridizing 12.5-kb species and a weakly hybridizing 15.4-kb species (Fig. 1 *A*, lanes *1* and *2*); both of which were clearly absent in DNA obtained from the patient after tumor remission (Fig. 1 *A*, lane *3*). Interestingly, hybridization with a probe (R28EX-4.9) (26, 27) from the TCR Dδ-Jδ region also detected a tumor-specific rearranged Bam HI fragment of 12.5 kb (Fig. 1 *B*).

To investigate the structure of these rearranged fragments, we constructed a λ phage library of Bam HI–digested leukemic DNA from patient 2114. Screening with the 11p13 bcr probe 2BE-2.4 yielded recombinant phage containing the rearranged 12.5-kb (λ12.5) or 15.4-kb (λ15.4) Bam HI fragments. Simultaneous screening with the chromosome 14 probe R28EX-4.9 revealed that the λ12.5 clones also annealed to DNA

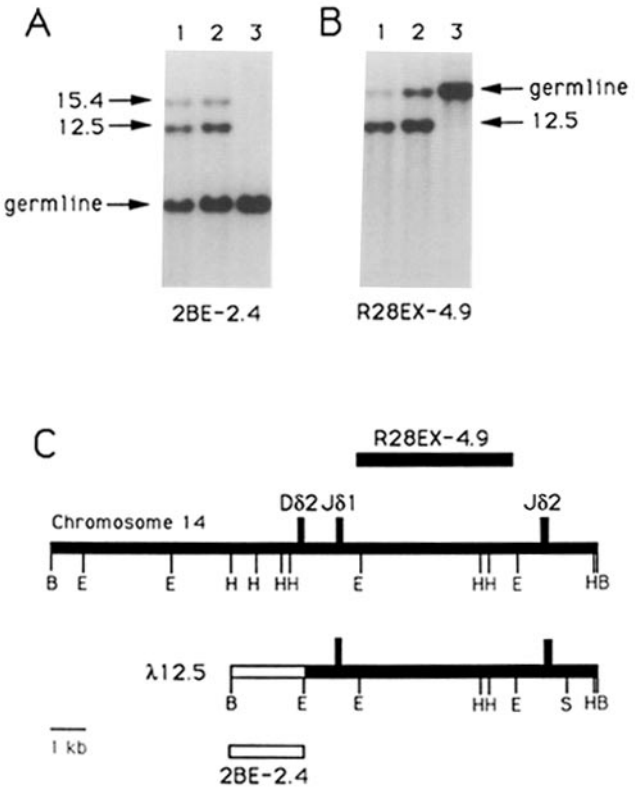

FIGURE 1. The translocation junction of chromsome $11p^+$ from patient 2114. Southern analysis of Bam HI–digested DNAs hybridized with probe 2BE-2.4 (*A*) or probe R28EX-4.9 (*B*). Lanes *1*, patient 2114 tumor sample 1; lanes *2*, patient 2114 tumor sample 2; lanes *3*, patient 2114 remission sample. (*C*) Recombinant clone λ12.5 was isolated from a phage library of patient 2114 tumor DNA with the 11p13-specific probe 2BE-2.4. The map of λ12.5 is compared with that of the Dδ-Jδ region of a germline TCR-α/δ chain gene. Closed boxes represent chromosome 14 sequences, and open boxes represent chromosome 11 sequences. Positions of the DNA probes 2BE-2.4 and R28EX-4.9 are indicated. Restriction sites: B, Bam HI; E, Eco RI; H, Hind III; S, Sac I (Sac I sites are not complete).

from the TCR Dδ-Jδ region. Hence, the rearranged 12.5-kb Bam HI fragment contains sequences derived from both 11p13 and 14q11, and therefore is likely to constitute the junction of a t(11;14)(p13;q11) chromosone translocation. This was confirmed by restriction mapping of the λ12.5 insert DNA, which shows it to be comprised of chromosome 11 sequences adjacent to the 11p13 bcr and chromosomc 14 sequences downstream of the TCR Dδ2 gene segment (Fig. 1 *B*).

Both Patients Share Identical Translocation Junctions on Chromosome 11p⁺. The t(11;14)(p13;q11) translocation generates two abnormal chromosomes, designated 11p⁺ and 14q⁻. From the known orientation of the TCR-α/δ chain gene on chromosome 14, we can deduce that phage clone λ12.5 from patient 2114 represents the t(11;14)(p13;q11) junction on chromosome 11p⁺. It is intriguing that the restriction map of λ12.5 (Fig. 1 *C*) is very similar to that of the 11p⁺ chromosome junction from patient 1114 (17). Nucleotide sequence analysis of λ12.5 reveals that the 11p⁺ junctions of these patients are not merely similar, but are in fact identical (Fig. 2, *B* and *C*), both resulting from rearrangement between the downstream recombination signal of Dδ2 (Fig. 2 *A*) and a pseudo recombination signal in the 11p13 bcr (Fig. 2 *D*).

The observation of identical translocation junctions in tumors from different patients is unprecedented. Therefore, it is necessary to address the possibility of cross-contamination between the corresponding clones from patient 2114 (λ12.5) and patient 1114 (λ2). Fortunately, these clones could be distinguished by a polymorphic Sac I restriction site located ~1 kb downstream of the TCR Jδ2 gene segment (26); restriction analysis reveals that λ12.5 contains the Sac I site (Fig. 1 *C*), whereas λ2 does not (data not shown). Southern analysis of Sac I-digested constitutional DNA indicates that patient 2114 is homozygous for the presence of the Sac I site, whereas patient 1114 is homozygous for its absence (data not shown). These data confirm the proper origin of λ12.5 and λ2 from patients 2114 and 1114, respectively, and thereby

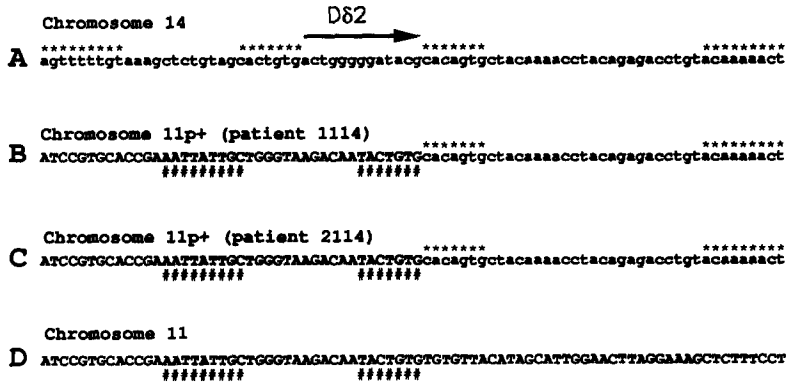

FIGURE 2. The chromosome 11p⁺ junctions resemble Ig/TCR signal joints. Comparison of (*A*) chromosome 14 sequences around the Dδ2 gene segment (14, 17); (*B*) the 11p⁺ translocation junction from patient 2114; (*C*) the 11p⁺ translocation junction from patient 1114; and (*D*) chromosome 11 sequences from the 11p13 bcr (17). Sequences derived from chromosome 14 are in lowercase letters, and those derived from chromosome 11 are in uppercase letters. The conserved heptamer and nonamer elements of the Dδ2 recombination signals are marked with asterisks, while those of the 11p13 pseudo recombination signal are marked with number symbols.

validate an observation that at first glance appears improbable: two unrelated T-ALL patients in the Dallas-Fort Worth area both underwent t(11;14)(p13;q11) translocation by somatically acquired, but seemingly identical, chromosome rearrangements.

Diversification of the Translocation Junctions on Chromosomes 14q⁻. It is intriguing that the 11p⁺ junctions from both patients result from precise fusions of the involved recombination signals, with neither loss or gain of nucleotides at the recombination site. As such, the 11p⁺ junctions resemble signal joints generated during normal Ig/TCR gene rearrangement. Therefore, if the t(11;14)(p13;q11) translocations were indeed mediated by the Ig/TCR recombinase, then we might expect the reciprocal 14q⁻ junctions to exhibit the random diversification typical of Ig/TCR coding joints. To investigate this possibility, we sought to isolate the 14q⁻ junction of patient 1114 by screening a λ phage library of Xba I-digested leukemic DNA with W3XS-0.7, a DNA probe from the 11p13 bcr (see Fig. 3). Hybridizing clones obtained in this manner are of two distinct types. Clones of one type contain the germline 11.9-kb Xba I fragment from chromosome 11. In contrast, clones of the other type (e.g., λH2XX; Fig. 3) contain a rearranged 12.0-kb Xba I fragment whose restriction pattern diverges from that of chromosome 11 in the vicinity of the 11p13 bcr. Nucleotide sequence analysis across the point of divergence reveals sequences of the TCR Dδ1 gene segment juxtaposed with sequences adjacent to the pseudo recombination signal of 11p13 (Fig. 4); hence, λH2XX represents the 14q⁻ translocation junction.

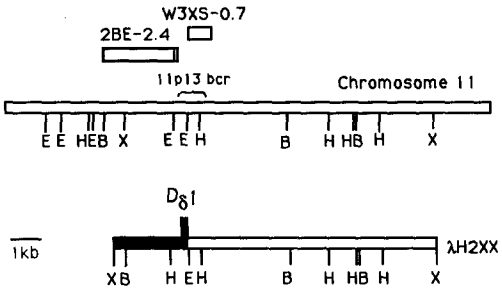

FIGURE 3. Isolation of the translocation junction of chromosome 14q⁻ from patient 2114. Recombinant clone λH2XX was isolated from a phage library of patient 2114 tumor DNA with the 11p13-specific probe W3XS-0.7. The map of λH2XX is compared with that of chromosome 11 in the vicinity of the 11p13 breakpoint cluster region (11p13 bcr). Closed boxes represent chromosome 14 sequences, and open boxes represent chromosome 11 sequences. The positions of DNA probes W3XS-0.7 and 2BE-2.4 are indicated. Restriction sites: B, Bam HI; E, Eco RI; H, Hind III; X, Xba I (Xba I sites are not complete).

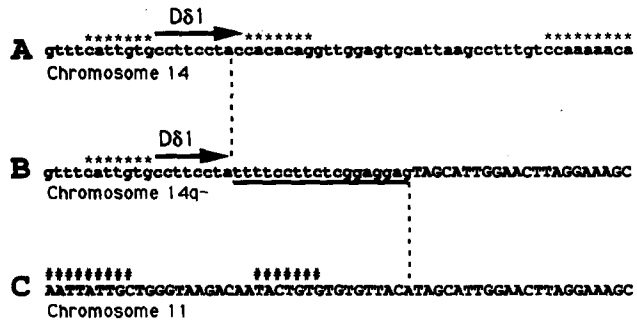

FIGURE 4. Diversification of the chromosome 14q⁻ junction from patient 2114. Comparison of (*A*) chromosome 14 sequences around the Dδ1 gene segment (41, 42); (*B*) the 14q⁻ translocation junction from patient 2114; and (*C*) chromosome 11 sequences from the 11p13 bcr (17). Nucleotides in uppercase letters are derived from chromosome 11, and those in lowercase letters are derived from chromosome 14 (excepting nucleotides generated by N-region insertion, which are indicated by underlining). The conserved heptamer and nonamer elements of the Dδ1 recombination signals are marked with asterisks, while those of the 11p13 pseudo recombination signal are marked with number symbols.

Moreover, this junction has undergone diversification due to the loss of a single nucleotide from the 3′ end of Dδ1, the random addition of 18 nucleotides at the recombination site, and the loss of nine residues from the 11p13 sequence. Clearly, t(11;14)(p13;q11) formation in patient 2114 involved a two-step process, since the 11p13 pseudo recombination signal participated in rearrangements with the downstream signals of both Dδ1 and Dδ2. As illustrated in Fig. 5, it is likely that the translocation arose by recombination between the 11p13 signal and the Dδ1 downstream signal, with formation of the diversified $14q^-$ junction described above (Fig. 5 *C*) and fusion of the responsible recombination signals at the $11p^+$ junction (Fig. 5 *D*). The original junction was deleted from chromosome $11p^+$, however, by subsequent recombination between the 11p13 signal and the Dδ2 downstream signal (Fig. 5 *E*). These tandem rearrangements bear the hallmarks of Ig/TCR recombinase activity, in that coding joints (exemplified by the $14q^-$ junction) are diversified, while signal joints (e.g., the $11p^+$ junction) are not. Unfortunately, strict asymmetry of junctional diversity cannot be proven, since the $11p^+$ and $14q^-$ junctions of this patient are not direct reciprocals of the same recombination event.

Southern analysis with an 11p13 bcr probe (W3XS-0.7) identified rearranged fragments in leukemic DNA from patient 1114 that were similar in size to those encompassing the $14q^-$ junction of patient 2114 (data not shown). Therefore, we isolated the $14q^-$ junction of patient 1114 by PCR with oligonucleotide primers that flank the junction of patient 2114; i.e., a 24 mer complementary to sequences upstream of Dδ1, and a 23 mer complementary to sequences adjacent to the 11p13 pseudo recombination signal. Analysis of the reaction product reveals sequences of Dδ1 and Dδ2 juxtaposed with sequences from 11p13 (Fig. 6). Thus, the Dδ1 and Dδ2 segments have recombined in a manner reminiscent of normal Dδ1 and Dδ2 rearrangments

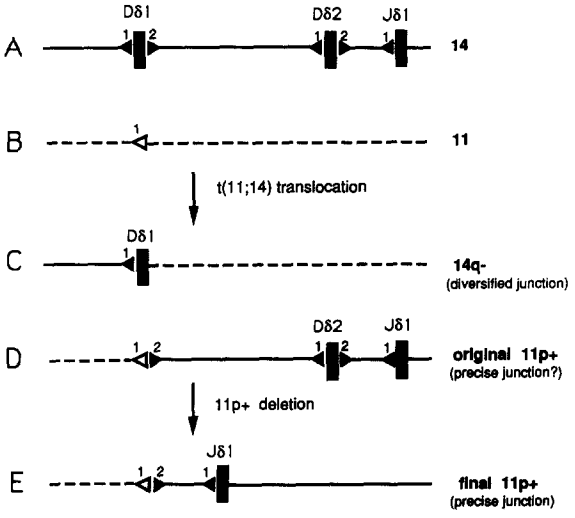

FIGURE 5. Schematic representation of the t(11;14) (p13;q11) translocation of patient 2114. This translocation was mediated by the downstream recombination signal of Dδ1 on chromosome 14 (*A*) and the 11p13 pseudo recombination signal on chromosome 11 (*B*). The translocation junction on the resultant $14q^-$ chromosome was diversified (*C*), whereas the original junction on the $11p^+$ chromosome presumably was not (*D*). Subsequent recombination between the 11p13 signal and the Dδ2 downstream signal generated the precise junction ultimately observed on $11p^+$ (*E*). Sequences from chromosome 14 and 11 are indicated by closed and dashed lines, respectively. The rectangles represent coding sequences of the TCR gene segments, and the triangles represent the associated recombination signals. The 11p13 pseudo recombination signal is indicated by an open triangle. The number over each recombination signal designates whether it possesses a one-turn (i.e., 12-bp) or two-turn (23-bp) spacer.

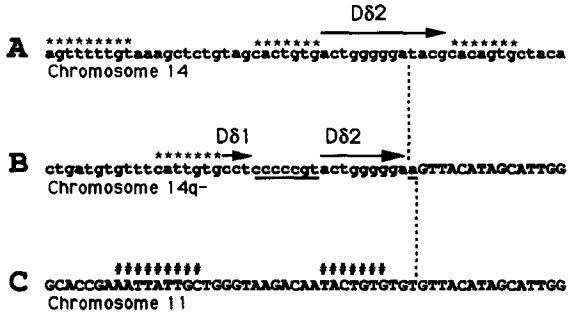

FIGURE 6. Diversification of the chromosome 14q⁻ junction from patient 1114. Comparison of (*A*) chromosome 14 sequences around the Dδ2 gene segment (14, 17); (*B*) the 14q⁻ translocation junction from patient 1114; and (*C*) chromosome 11 sequences from the 11p13 bcr (17). Nucleotides in uppercase letters are derived from chromosome 11, and those in lowercase letters are derived from chromosome 14 (excepting the adenosine residue generated by N-region insertion, which is indicated by underlining). The conserved heptamer and nonamer elements of the Dδ recombination signals are marked with asterisks, while those of the 11p13 pseudo recombination signal are marked with number symbols.

(see Fig. 7); in particular, junctional diversification has occurred due to loss of six nucleotides from the 3′ end of Dδ1 and the random addition of seven nucleotides at the recombination site (Fig. 6). More interestingly, however, diversification is also apparent at the translocation junction: four nucleotides are lost from the 3′ end of Dδ2, a single adenosine residue is added at the recombination site, and three nucleotides are lost from 11p13 (Fig. 6). Furthermore, the 11p⁺ and 14q⁻ junctions of patient 1114 are reciprocal products of the same recombination event, both arising due to rearrangement between the 11p13 signal and the Dδ2 downstream signal (Fig. 7). Hence, the junctional diversity engendered during t(11;14)(p13;q11) translocation in patient 1114 is confined to the 14q⁻ junction, and thereby exhibits the strict asymmetry typical of Ig/TCR gene rearrangements.

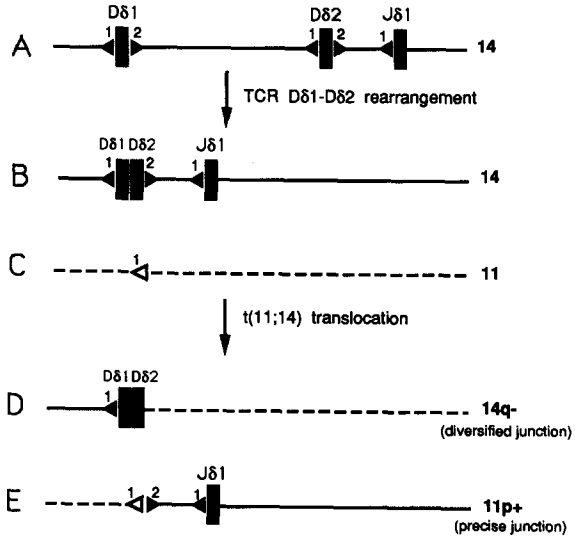

FIGURE 7. Schematic representation of the t(11;14)(p13;q11) translocation of patient 1114. The involved chromosome 14 had undergone local rearrangement of its Dδ1 and Dδ2 gene segments (*B*). The Dδ1-Dδ2 rearrangement could have occurred before (as illustrated) or after the chromosome translocation. In either case, the t(11;14)(p13;q11) translocation arose from rearrangement between the downstream recombination signal of Dδ2 on chromosome 14 (*B*) and the 11p13 pseudo recombination signal on chromosome 11 (*C*). This rearrangement generated a diversified translocation junction on chromosome 14q⁻ (*D*) and a precise junction on 11p⁺ (*E*).

Continuing DNA Rearrangement within the Translocated TCR Allele during Tumor Expansion. Southern hybridization of tumor DNA from patient 2114 with the 11p13 bcr probe identified a rearranged 15.4-kb Bam HI fragment in addition to the 12.5-kb fragment encompassing the translocation junction of $11p^+$ (Fig. 1 *A*). Restriction mapping of a recombinant λ phage clone (λ15.4) containing the 15.4-kb fragment revealed it to be comprised of 11p13 DNA juxtaposed with sequences downstream of the TCR Jδ3 gene segment (Fig. 8 *A*). Indeed, nucleotide sequence analysis of λ15.4 identified sequences of the 11p13 pseudo recombination signal fused to sequences of Jδ3, with random addition of nine nucleotides at the junction (Fig. 8 *B*). Thus, patient 2114 harbored two populations of leukemic cells, one in which 11p13 DNA translocated into the TCR locus at a site near the Dδ2 gene segment (as represented by λ12.5), and another in which 11p13 DNA translocated near the Jδ3 segment. It is unlikely that these populations arose independently, especially as there is no evidence of biclonality upon Southern hybridization with TCR-β or -γ gene probes. Instead, the λ12.5 rearrangement probably represents the $11p^+$ junction in the initial population of transformed cells; the λ15.4 rearrangement presumably arose at a subsequent stage of tumor expansion as a result of recombination within the $11p^+$ chromosome between the Dδ2 downstream recombination signal and the Jδ3 signal. During this event, the 11p13 signal of λ12.5 would be recognized by the recombinase as coding sequences; hence, the 11p13 signal itself was subjected to junctional diversification, as demonstrated by the loss of six nucleotides from its heptamer in λ15.4 (Fig. 8 *B*).

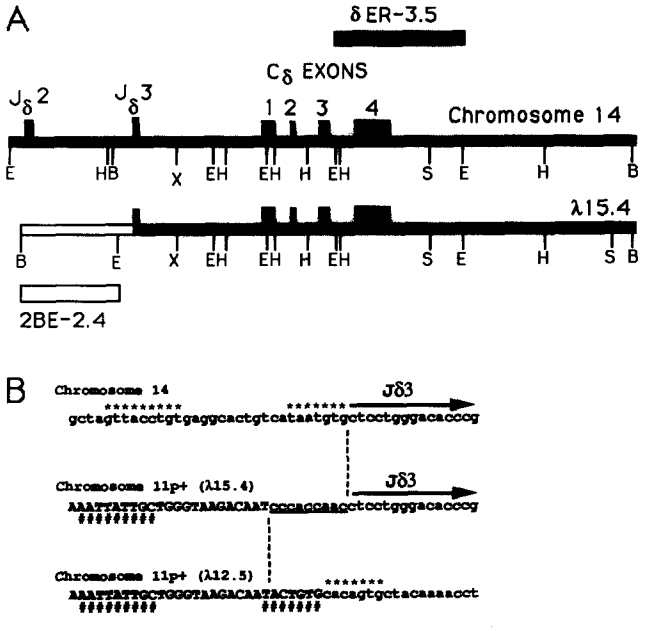

FIGURE 8. Continuing rearrangement of the translocated TCR-α/δ chain allele. (*A*) Recombinant clone λ15.4 was isolated from a phage library of patient 2114 tumor DNA with the 11p13-specific probe 2BE-2.4. The map of λ15.4 is compared with that of the Jδ-Cδ region of a germline TCR α/δ chain gene. Closed boxes represent chromosome 14 sequences, and open boxes represent chromosome 11 sequences. Restriction sites: B, Bam HI; E, Eco RI; H, Hind III. (*B*) Comparison of chromosome 14 sequences around the Jδ3 gene segment (43), the $11p^+$ junction from λ15.4, and the $11p^+$ junction from λ12.5. Nucleotides in uppercase letters are derived from chromosome 11, and those in lowercase letters are derived from chromosome 14 (excepting nucleotides generated by N-region insertion, which are indicated by underlining). The conserved heptamer and nonamer elements of the Jδ3 recombination signal are marked with asterisks, as is the heptamer element of the Dδ2 downstream signal. The heptamer and nonamer elements of the 11p13 pseudo recombination signal are marked with number symbols.

Discussion

Chromosome abnormalities associated with lymphoid tumors frequently feature cytogenetic breakage within the Ig or TCR genes (reviewed in reference 2). Typically, these arise due to recombination between two disparate loci, only one of which is an Ig/TCR gene. In some cases the other participating locus has been shown to be a proto-oncogene, the expression of which is altered in a manner that presumably promotes tumor development. Although the recurrence of particular chromosome defects in lymphoid neoplasms is likely to be a consequence of tumor selection, their formation has been ascribed to rare aberrant activity of the Ig/TCR recombinase. This is supported by the fact that breakpoints within the Ig/TCR loci usually occur adjacent to recombination signals. On the other hand, sequences bearing convincing homology to recombination signals are only seldomly observed at the breakpoints of the other participating loci. Thus, two models have emerged to describe the formation of tumor-specific chromosome abnormalities involving the Ig/TCR loci (28–30). Both models implicate the recombinase in site-specific scission of the Ig/TCR gene. Presumably, the recombinase also mediates reciprocal religation of the broken DNA ends from the Ig/TCR locus with the broken ends from a second chromosomal locus. The two models differ, however, in the mechanism of scission at that second chromosomal locus. In one model, scission is mediated by the recombinase upon recognition of a sequence with fortuitous homology with genuine Ig/TCR recombination signals (28, 30). In the other model, scission is achieved independent of the recombinase, either randomly or catalyzed by distinct endonucleolytic activities (29).

A striking example of the first model was reported by Tycko et al. (31) in their analysis of t(7;9)(q34;q32), a recurrent translocation associated with T-ALL; in each of three patients, chromosome 7 breakage occurred adjacent to a TCR Jβ gene segment, while chromosome 9 breaks were clustered between two sequences with identity to the consensus heptamer of true recombination signals. Although the pseudo recombination signal on chromosome 9 has a configuration (heptamer-space-heptamer) different from that of normal recombination signals (heptamer-space-nonamer), the data imply that the signal recognition and DNA scission activities of the Ig/TCR recombinase promote cleavage of both chromosomes 7 and 9 before t(7;9)(q34;q32) formation. Conversely, however, in their study of the t(14;18) (q32;q21) translocation associated with follicular B cell lymphoma, Bakhshi et al. (29) noted the absence of pseudo recombination signals in the 150-bp region wherein most of the chromosome 18 breakpoints occur. Indeed, examination of the reciprocal products of translocation in one patient revealed a direct repeat duplication of chromosome 18 sequences at the junctions of both the der(14) and der(18) chromosomes. The duplication presumably arose from repair of a staggered double-stranded break on chromosome 18. Thus, an alternative model of t(14;18)(q32;q34) formation was proposed, whereby flush cleavage at an Ig J_H gene segment of chromosome 14 was mediated by the Ig/TCR recombinase, while the staggered double-stranded break on chromosome 18 arose independent of recombinase activity.

During normal lymphoid development, the Ig and TCR genes rearrange to join V, D, and J segments into a contiguous exon encoding the variable domain of an Ig or TCR polypeptide (3–5). Several lines of evidence underscore the asymmetric nature of Ig/TCR gene rearrangement. Examination of the reciprocal products of recombination reveals that whereas signal joints are usually precise, coding joints

are diversified by random loss and addition of nucleotides at the recombination site (32–35). Moreover, a mechanistic asymmetry is implied by the *scid* mutation, which prevents coding joint formation without seriously impairing formation of signal joints (36, 37). The asymmetry may reflect the fact that the sequences that form coding joints are variable, whereas those forming signal joints are conserved and specifically recognized by proteins likely to be associated with the recombinase complex (38). Indeed, on the basis of their study of V_L-J_L formation in chicken bursae, McCormack et al. (39) proposed that site-specific scission by the recombinase generates an intermediate structure in which the signal ends are physically bound to the recombinase complex. Thus, in the interval before religation, signal ends would be protected from the enzymatic processes that diversify coding ends. If this is indeed the case, then asymmetric diversification should also be a characteristic of particular tumor-specific chromosome rearrangements; i.e., those in which scission at both participating loci were catalyzed by the Ig/TCR recombinase.

Our analyses of t(11;14)(p13;q11) formation in patients 2114 and 1114 clearly implicate the Ig/TCR recombinase in the scission of both chromosomes 11 and 14. Notably, the chromosome 11 breakpoints occur immediately adjacent to the 11p13 pseudo recombination signal. Moreover, examination of the translocation junctions reveals an asymmetric pattern of diversification typical of that generated during normal Ig/TCR gene rearrangement. In each case, the $11p^+$ chromosome junction resembles a signal joint in that it is comprised of a precise fusion of the 11p13 pseudo recombination signal and the downstream Dδ2 recombination signal. In contrast, the reciprocal junctions on $14q^-$ are diversified by random loss and gain of nucleotides in a fashion reminiscent of the coding joints generated during normal Ig/TCR gene rearrangement.

Junctional diversification has been observed in other chromosomal rearrangements of lymphoid tumors (2). However, the asymmetric nature of the process has not been apparent, because in most studies only one of the two reciprocal products was characterized. The t(7;9)(q34;q32) translocation would be expected to exhibit asymmetric diversification, since its formation, like that of the t(11;14)(p13;q11) translocations described here, proceeds upon recombinase-mediated scission of both participating chromosomes. Nonetheless, Tycko et al. (31) observed diversification of both t(7;9)(q34;q32) junctions. Formation of t(7;9)(q34;q32) is complex, with concurrent deletion of intervening sequences between the junctional Dβ and Jβ segments, including the recombination signals associated with these segments. Hence, the final t(7;9) (q34;q32) junctions are unlikely to represent both products of the original translocation event. In contrast, the $11p^+$ and $14q^-$ junctions of patient 1114 clearly correspond to the original products of t(11;14)(p13;q11) translocation, and thus provide a fortuitous observation of the asymmetry expected of recombinase-mediated DNA rearrangements.

Aberrant chromosomal rearrangments are likely to arise in a proportion of cell divisions during normal development. In cells with Ig/TCR recombinase activity, such rearrangements may preferably involve sites with homology to its recognition sequence, especially the Ig/TCR loci which harbor arrays of genuine recombination signals. Indeed, cytogenetic studies have identified chromosomal rearrangement between Ig/TCR loci in a demonstrable proportion ($\sim$0.2%) of mitogen-stimulated lymphocytes from normal individuals (40). However, the chromosomal rearrangements that recurrently emerge in tumors are probably those that promote neoplastic development. Accordingly, tumor selection dictates the breakpoint cluster regions

characteristic of each tumor-specific chromosome abnormality. The relevance, therefore, of the two models describing the formation of these abnormalities in lymphoid tumors probably reflects the presence or absence of pseudo recombination signals within the bcr. As noted, the major breakpoint region for t(14;14)(q32;q21) translocation on chromosome 18 is devoid of such sequences (29). On the other hand, the 11p13 bcr harbors a number of pseudo recombinations signals, and the t(11;14)(p13;q11) breakpoints do appear to be directed to these sites (14, 17). Thus, of the four t(11;14)(p13;q11) translocations analyzed to date, two involve the pseudo recombination signal described here, and a third breaks adjacent to a distinct heptamer-like sequence in the 11p13 bcr (14).

Summary

The t(11;14)(p13;q13) translocation associated with T cell acute lymphocytic leukemia generates two abnormal chromosomes, designated $11p^+$ and $14q^-$. To investigate the mechanism of t(11;14)(p13;q11) formation, we analyzed the translocation junctions of $11p^+$ and $14q^-$ from two patients. The $11p^+$ junctions consisted of precise fusions of a pseudo recombination signal from chromosome 11 and the downstream recombination signal of the TCR Dδ2 gene segment from chromosome 14. In contrast, the $14q^-$ junctions from both patients were diversified by random loss and addition of nucleotides at the translocation site. This asymmetric pattern of junctional diversification is typical of normal Ig/TCR gene rearrangement, and therefore implies that the t(11;14)(p13;q11) translocation arose due to aberrant activity of the Ig/TCR recombinase.

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