Novel Chromosome Translocation Caused by Fusion of Immunoglobulin Heavy and Light Chain V Genes in a Human B Lymphoblastoid Cell Line

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Summary

The chromosome breakpoints of a translocation, t(2;14), from an Epstein-Barr virus–transformed human B lymphoblastoid cell line were isolated and analyzed. This unusual translocation arose as a result of the fusion of two immunoglobulin (Ig) variable (V) genes, one from the heavy chain cluster on chromosome 14, the other from the light chain (κ) cluster on chromosome 2. The chromosome breaks occurred within the coding sequence of each gene, and there was no obvious evidence for lymphoid V(D)J recombinase involvement in the translocation. This suggests that breakage and rejoining of the involved V genes occurred by some process other than that which normally rearranges Ig genes.

Materials and Methods

Cell Line. JC11 was derived by EBV transformation of PBL from a patient with chronic myeloid leukemia. This cell line lacked the Ph' chromosome seen in the patient's leukemic cells, but contained as the sole abnormality a novel translocation t(2;14)(p11;q32) not present in the patient's blood or bone marrow cells (4).

DNA Isolation and Southern Blotting. DNA extraction and Southern blotting were carried out as previously described (4). The probe for Jκ was a 1.8-kb Sacl fragment derived from pJ-2 (a gift from Dr. G. Bornkamm, Institute for Virology, Freiburg, Germany).

Construction and Screening of Genomic DNA Libraries. Genomic DNA from JC11 was digested with EcoR1, and ligated to XZAP DNA (Stratagene, La Jolla, CA). Recombinant molecules were packaged in vitro, and then screened as described (5). The chromosome 14–specific genomic DNA library was obtained from the American Type Culture Collection, (57739; Rockville, MD).

DNA Sequencing. DNA sequences were established (7) using T7 DNA polymerase with plasmids propagated in host strain DH5α and prepared by rapid boiling lysis (8).

Results and Discussion

We have shown that the breakpoints of the JC11 translocation t(2;14) lie within the Ig VH gene cluster, and within or upstream of the IgL Jκ genes (4). Southern blots of JC11 DNA showed that both Jκ alleles were rearranged relative to DNA from nonlymphoid cells (Fig. 1 A). JC11 produces functional IgL(κ) chains and therefore one of the rearranged fragments represents a productively joined VκJκ allele (4).

Because Jκ genes are commonly involved in translocations with 2p11 breakpoints, it seemed possible that the other rearranged fragment resulted from the 14q- breakpoint of the t(2;14).

Molecular cloning was used to isolate the two EcoR1 fragments that contained rearranged Jκ sequences. Recombinant
Figure 1. Rearranged Jk alleles of JC11. (A) Southern blot of JC11 DNA (lane 1) and DNA from cells of a normal donor (lane 2) digested with EcoRl and hybridized with a Jk probe. Rearranged Jk alleles in JC11 DNA are arrowed. The germline (gl) band in lane 2 is ~10 kb in size. (B). Restriction maps of plasmids containing the rearranged EcoRl fragments from JC11 aligned with the gl Jk map. Regions that are noncontiguous with gl Jk are hatched. Black bars show Jk exons. Sequencing strategy is indicated by arrows below each clone. A, Apal; B, BamHI; Bg, BglII; Bs, BstEII; N, Ncol; Pvu, PvuII; Ps, PstI; RI, EcoRI; SI, Sall; SII, SacII; Sc, Scal.

plasmids containing the 5- and 3-kb EcoRl fragments (pCY15 and pCY16, respectively) were recovered and characterized (Fig. 1 B). The 3' map of both clones matched the germline (gl) Jk locus, but this similarity ceased 5' to Jk2 in pCY15, and 5' to Jk3 in pCY16. This suggested that both Jk alleles had undergone fusion with noncontiguous DNA. Probes from the 5' end of each isolate detected different multiple bands on Southern blots of digested human DNA, indicating that each came from a different family of multiple related sequences (data not shown). Hybridization of each probe to plaque-lifts prepared from a genomic chromosome 14-specific DNA library suggested that sequences in pCY16, but not pCY15 were derived from chromosome 14 (data not shown).

The structure of both rearranged Jk alleles was determined by DNA sequence analysis. The sequence of pCY15 (Fig. 2) revealed a fusion of Jk2 with a Vk gene very similar to V6410, a VxII family member (10). The pCY15 Vxjk sequence has a reading frame capable of translation into a Vx L chain, and this presumably represents the productive Ig L chain locus of JC11. Sequence analysis of pCY16 revealed a more complex structure (Fig. 3). Immediately 5' to Jk3 is a sequence that shows strong homology to part of a VKI gene (11). This suggests that a conventional recombinase-mediated fusion of Vx and Jk elements has occurred. However, the homology with VkI ceases abruptly 33 bp 5' to the VJ junction, within the framework (FR) III region of the Vx gene. Adjacent to the 5' end of the 32-bp Vx segment is a region that shows extensive homology to members of the VHI class of Ig H chain V region genes (11). This VH-like sequence is inverted with respect to the Vxjk region and it extends 5' from within CDR2 to the heptamer and nonamer RSS, which normally

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\begin{align*}
V_{K} & \quad TCTCAGATCCAGACAGCTGCCAGACGAGTCTCGACACATCTGCTGAGACGTTTCTGTACCTCACCAAGGACG
\end{align*}
\]
form the 3' flank of gl VH genes (Fig. 4). Homology with gl VH extends 5' of these signal sequences for 420 bp (data not shown), beyond which no further sequence data were obtained. Therefore, the structure of this 14q+ chromosome breakpoint shows evidence of two distinct gene fusion events. One appears to be a conventional V(DJ) recombinase-mediated fusion between Vλ and Jκ segments. The other fusion is novel, and apparently gave rise to the translocation, by joining Ig V region genes from two different chromosomes.

To confirm the nature of this translocation, the breakpoint from the 2p- chromosome was isolated. The structure of this breakpoint was expected to be the reciprocal product of the fusion between VH and VK at the 14q+ breakpoint, which represented a unique arrangement amenable to enzymatic amplification by PCR. Oligonucleotide primers complementary to two sites within VHI FR1 regions, and to Vκ1 FR2 and FR3 regions (Fig. 4), generated a 200-bp DNA fragment from JC11 DNA, but not from three unrelated B lymphoblastoid cell lines, when used in a nested PCR (data not shown). The DNA sequence of the JC11-specific fragment clearly showed that it was the reciprocal product of the rearrangement seen at the 14q+ breakpoint (Fig. 3).

A comparison of the sequences of both breakpoints with each other and with closely related Ig V genes (Fig. 3) reveals several features. Although the presence of the sequence “GAT” on both chromosomes 2 and 14 means the breakpoint cannot be unambiguously assigned, it seems likely that the VH gene has not lost any nucleotides during translocation. In contrast, 11 nucleotides of the VK gene appear to have been lost during the translocation process (although in the absence of a gl sequence for this gene the precise extent of loss cannot be determined, nor can the prior existence of a deletion at this position be excluded). These observations suggest that during translocation the VH gene suffered a double-stranded DNA break, whereas the VK gene was cleaved by two staggered single-stranded breaks several nucleotides apart. Exonuclease-mediated removal of the single-stranded extensions could account for the proposed loss of nucleotides seen at the breakpoint. No significant matches with the consensus RSS could be found in the immediate vicinity of the breakpoints, which argues against — but does not formally exclude — the involvement of Ig gene fusion mechanisms in the translocation.

This t(2;14) is the first characterized example of an inter-chromosomal fusion between Ig genes. A previously reported t(2;14) involved H chain Ig genes but not L chain genes (12). Chromosome rearrangements that lead to the precise fusion of Ig and TCR elements from different clusters presumably result from recombinase activity (1, 3). In contrast, the inter-chromosomal fusion of Ig V genes described here does not occur at RSS, and it more closely resembles the product of nonspecific breakage and rejoicing. Other examples of lymphoid translocations exist in which the breakpoints do not occur at RSS, and in some cases, like the t(2;14) of JC11, breakpoints lie within Ig V genes (1, 3). Therefore, aberrant recombinase activity cannot easily account for a significant number of lymphoid translocations, including this t(2;14), and alternative mechanisms must be considered.

Random DNA breakage and repair by endogenous ligase activity could account for the structure of the t(2;14). Significantly, the patient from whom JC11 was derived had a 16-yr history of treatment with busulfan, an alkylating agent known to induce chromosomal abnormalities (13). PHA-stimulated PBL from this patient showed nonclonal chromosomal aberrations typical of busulfan-induced damage (Dr. P. Hollings, personal communication). Therefore, it is possible that DNA damage caused by the patient's chemotherapy may have contributed to development of the t(2;14). However, if this B lymphoid translocation did not result from aberrant V(DJ) recombinase activity, why have two gene clusters expressed exclusively in B cells been involved? One explanation could be that those conformational features of expressed Ig genes that are proposed to allow access by recombinase (14) also increase susceptibility of DNA in these regions to damage. The 14q+ breakpoint of this t(2;14) occurred within the VH cluster of the productively rearranged Ig H chain allele (4), and the break on chromosome 2 occurred near a Vκ Jκ rearrangement (Fig. 4). Therefore, both regions involved in the translocation were presumably in an “open” or accessible state during development of the cell that gave rise to JC11. Genomic DNA in this state could conceivably be more exposed to the effects of DNA-damaging agents (such as busulfan) than DNA in regions of “closed” or inaccessible chromatin fibers. Repair of DNA breaks could be mediated by endogenous ligase activity or, alternatively, it is possible that the V(DJ) recombinase can preferentially fuse antigen receptor genes that suffer spurious double-stranded DNA breaks. This model is compatible with the observation that translocations in various cell types tend to occur in regions of the genome encoding differentiation-specific products (15).

Regardless of the precise mechanism involved, the breakpoints described here demonstrate that Ig genes may participate in chromosome translocations of B cells without obvious involvement of the V(DJ) recombinase system. This
may be related to the conformationally "open" state, which antigen receptor genes are thought to adopt during lymphoid cell development (14). The derivation of the JC11 cell line from a patient with a long history of treatment by an alkylating agent prompts speculation that DNA damaging agents may preferentially affect "accessible" DNA loci, thus promoting some lymphoid translocations. This proposed effect delineates a role for DNA damaging agents in some lymphoid translocations, which is difficult to accommodate in a model exclusively reliant on aberrant activity of the V(D)J recombinase.

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