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Molecular Characterization of Novel Reciprocal Translocation t(6;14) in an Epstein-Barr Virus-Transformed B Cell Precursor

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An in vitro culture of FLEB14 cells, an Epstein-Barr virus-transformed B cell precursor containing the germ line immunoglobulin genes, gave rise to a uniclonally expanded variant, FLEB14Δ3, which was rearranged at the immunoglobulin heavy-chain gene locus. Cytogenetic analysis showed that FLEB14Δ3 had a novel reciprocal translocation, t(6;14)(q15;q32). Molecular cloning of the rearranged DNA fragments and determination of their nucleotide sequence revealed that the recombination event was reciprocal, imprecise, and nonhomologous and took place in the Sμ region, like those found in Burkitt's lymphoma cells. We propose a molecular model to explain this genetic event which may be relevant to class switch recombination. The translocated sequence of chromosome 6 did not contain any known oncogenes, although the sequence is conserved among mammals. FLEB14Δ3 did not show tumorigenicity.

Epstein-Barr virus (EBV) is the virus isolated from Burkitt's lymphoma, a B cell malignancy (for a review see reference 36). In in vitro experiments, EBV infects specifically B cells of humans and some primates, leading them to permanent proliferation. Burkitt's lymphoma is highly endemic in central Africa and New Guinea, where patients with Burkitt's lymphoma show higher titers against EBV antigens. Burkitt's cells established from these areas usually contain the EBV genome. These facts have long indicated that EBV is the possible causative agent of Burkitt's lymphoma.

EBV-transformed cells, however, show different features from those found in Burkitt's cells. B cells immortalized by EBV grow as morphologically heterogeneous and clustered cells, while Burkitt's lymphoma cells grow as morphologically homogeneous and isolated cells. In particular, EBV transformants do not have either high plating efficiency or tumorigenicity, which are markers of malignancy. The Burkitt's lymphomas that are rarely found in areas such as Europe, the United States, and Japan often lack the EBV genome. Furthermore, it is well known that Burkitt's lymphoma cells, regardless of the presence of the EBV genome, show frequent chromosomal translocations involving chromosome 8: t(8;14), t(2;8) and t(8;22) (28, 36). Such chromosomal aberrations were suggested to be related to oncogenesis in Burkitt's cells (24, 25). Recent analyses at the molecular level have confirmed this hypothesis and revealed that the rearrangement occurred between the c-*myc* gene on chromosome 8 (8q24) and the immunoglobulin heavy (H)-chain gene on chromosome 14 (14q32), the kappa-chain gene on chromosome 2 (2p13), and the lambda-chain gene on chromosome 22 (22q11) (1, 4, 29, 48). On the other hand, EBV transformants have not so far been reported to have such translocations. The results have made it uncertain whether EBV is indeed involved in oncogenesis in Burkitt's cells.

Differentiation stages of B lymphocytes are clearly defined by the profiles of the immunoglobulin gene rearrangement (14). The initial event is D_H-J_H recombination at the H-chain locus (pro-B cell), followed by completion of a V_H-D_H-J_H complex (pre-B cell). The pre-B cell produces the μ chain in the cytoplasm. The light (L)-chain locus then undergoes V_L-J_L recombination to produce a complete immunoglobulin composed of the H and L chains. The B cell thus formed expresses the immunoglobulin on the surface. Subsequently, the S-S recombination mediated by the repetitive intervening sequences (switch regions) lying between the V_H and C_H genes (44) takes place to switch classes of the immunoglobulin.

Since EBV can transform and freeze B cells at respective stages of the human B cell lineage, EBV is a useful tool for studying molecular events of human B cell differentiation (36). We have recently shown that EBV is able to transform B cells at a very early stage that have no rearrangement of the immunoglobulin genes (19). This stage of the B cell was called the pre-pro-B cell (14). Subsequent culture of such a cell line, FLEB14, resulted in production of a spontaneous variant, FLEB14Δ3, that had a distinct rearrangement at the J_H locus. FLEB14Δ3 had a reciprocal translocation, t(6;14)(q15;q32). We have studied the structures of the recombination regions and found that the Sμ region is involved. This study clearly demonstrates that the EBV transformant undergoes recombination in a region similar to those in Burkitt's cells. Furthermore, we present a molecular model of the recombination which took place in FLEB14Δ3.

MATERIALS AND METHODS

Cell lines. The FLEB14Δ3 line used in this study was a derivative of FLEB14 line which was established from a

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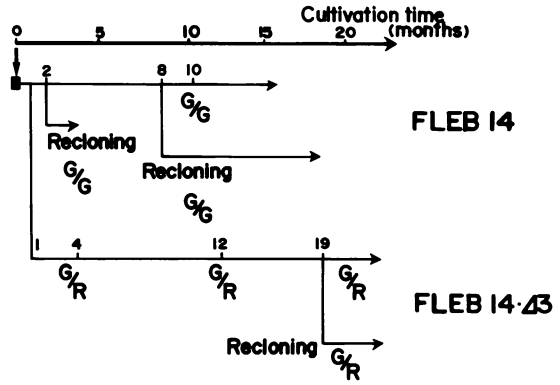

FIG. 1. Immunoglobulin H-chain gene organizations of the FLEB14 and FLEB14Δ3 lines at various time intervals. The immunoglobulin H-chain gene organization of one allele is denoted by G and R, which indicate the germ line and rearranged states, respectively. G and R were determined by Southern blot analysis (Fig. 3). FLEB14 was an immature B-cell line without rearrangement of immunoglobulin genes. Sublines of FLEB14 cloned 2 and 8 months after establishment conserved this germ line gene organization. One month after establishment, FLEB14 was distributed to another collaborator and continuously cultivated independently. From this FLEB14 line, the FLEB14Δ3 line was spontaneously isolated and identified as a line whose immunoglobulin H-chain gene is rearranged in one allele (G/R). The state of G/R was observed at the latest 4 months after establishment. Sublines of the FLEB14Δ3 line also showed the G/R pattern 19 months after establishment. Lines with arrows indicate sublines. Numbers indicate months after establishment of FLEB14.

human fetal liver at week 16 of gestation by EBV transformation (19). As already characterized, FLEB14 is a cell line with B cell phenotypes but without immunoglobulin gene rearrangement. These two lines were successively cultivated in RPMI 1640 medium with 20% fetal calf serum, streptomycin, and ampicillin. The cloning of their sublines was performed by the soft agar colony formation method (51). To confirm the chromosomal localization of the rearranged fragment, we used a mouse × human hybrid, MCP-6, which retains only a human t(6;X)(6p21→6qter::Xq13→Xqter) chromosome (8). As controls, mouse × human hybrid 1W1-5, containing only human Xq11-Xqter (16), in addition to parental mouse cell lines PCC4 and 1RE3 (8) were used.

Biological characterization. Cytoplasmic and surface immunoglobulins were detected by direct immunofluorescence. EBNA (EBV-induced nuclear antigen) was studied by the method of Reedman and Klein (39). B cell antigens (B4, B1, B2, BB-1, and 3A8B) and CALLA (J5) were tested by indirect immunofluorescence with fluoroscein isothiocyanate-labeled rabbit anti-mouse immunoglobulin antibodies (Dako) and scored after counting 300 cells. T cell antigens (Leu-1, -2a, -3a, and -4) were analyzed by fluorocytometry as described previously (19). Chromosomes were studied by a modification of trypsin-Giemsa banding method of Seabright (42). At least 20 metaphase spreads were examined for each cell line, and well-delineated metaphases were photographed for karyotype analysis. Tumorigenicity of cell lines was examined by their ability to form growing masses after subcutaneous inoculation into mice (33). CBA mice thymectomized 5 to 9 weeks after birth were treated with cytosine arabinoside (0.2 mg/g of mouse weight) 3 weeks later, irradiated with X-ray at 735 rads 3 days later, and then inoculated subcutaneously with 2×10^7 cells of each cell line per mouse. Tumor formation was observed for at least 60 days and scored.

Southern blot analyses of genomic DNAs. High-molecular-weight DNAs were prepared from human placenta, FLEB14, and FLEB14Δ3 cells (3, 53). DNAs were digested with restriction enzymes, electrophoresed in 0.5% agarose gels, and transferred to nitrocellulose filters by the method of Southern (45). The DNAs on filters were hybridized with appropriate probes labeled by nick translation as described previously (15) or with a labeling kit (Amersham). Following hybridization at 65°C for 14 to 16 h, filters were washed three times at 65°C in 0.1× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate)–0.1% sodium dodecyl sulfate (SDS) and then autoradiographed. The probes used for immunoglobulin gene analysis were as follows: J_H, 3.5-kilobase (kb) *Eco*RI-*Hin*dIII fragment (47); Cμ, 1.2-kb *Eco*RI-*Eco*RI fragment (47); 5′C_μ, 1.0-kb *Sac*I-*Eco*RI fragment (38); J_κ, 2.0-kb *Sac*I-*Sac*I fragment (12; unpublished data); Cλ, 4.0-kb *Eco*RI-*Hin*dIII fragment (11, 38). The evolutional conservation of the sequence in question was analyzed by using high-molecular-weight DNA from the livers of other species.

Cloning and sequencing procedures. Molecular cloning of the germ line J_H fragment of FLEB14 as well as the rearranged J_H and 5′C_μ fragments of FLEB14Δ3 line were performed as described previously (20) with Charon 4A or λgtWES vectors (26). The recombinant phages were screened by the method of Benton and Davis (2). The probes used were the J_H and 5′C_μ fragments mentioned above. A Charon 4A library containing *Eco*RI*-digested DNA from the human immunoglobulin E (IgE)-producing cell line 266BL (37) was also used. The DNA sequences were determined by the dideoxy DNA sequencing method (41) with plasmid vectors pUC18 and pUC19 as described previously (10). Primers and sequence kits were purchased from Takara Shuzo, Co. Ltd. (Kyoto) or Pharmacia Japan (Tokyo).

RESULTS

Establishment of FLEB14Δ3 line. The FLEB14 cell line is a B cell line of the most immature stage which had no immunoglobulin gene rearrangement, as described previously (19). FLEB14 cells retained the germ line configuration of the H-chain locus during cultivation for more than 3 years, which was confirmed by immunoglobulin gene analyses of the cloned sublines (Fig. 1). We attempted to promote immunoglobulin gene rearrangement in FLEB14 by the addition of various lectins or chemicals, without success. During the course of this study we found that a subline of FLEB14, FLEB14Δ3, underwent spontaneous rearrangement in the H-chain gene. This rearrangement was noticed 4 months after establishment of the FLEB14 line.

Phenotypic and karyotypic characterization of FLEB14Δ3 line. FLEB14Δ3 cells had phenotypes similar to FLEB14, as shown in Table 1. Although neither of them expressed membrane-bound or cytoplasmic immunoglobulin, both possessed antigens characteristic of B cells (B4, B1, B2, and BB-1). Since 3A8B, an antigen frequently positive in Burkitt's cells but negative in EBV transformants, was not detected, FLEB14 and FLEB14Δ3 cells had phenotypes characteristic of EBV transformants. EBNA was detected in both cases, in agreement with the virus infection. T-cell antigens were negative in the FLEB14 line (19). These characterizations agreed with the previous conclusion (14, 19) that FLEB14 was in a B cell lineage, *i.e.*, pre-pro-B cell.

Karyotype analysis revealed that while the FLEB14 line retained the normal karyotype of 46,XY, its derivative line

FLEB14Δ3 showed a reciprocal translocation between chromosomes 6 and 14, represented by 46,XY,t(6;14)(q15;q32) (Fig. 2). As a result of the translocation, two marker chromosomes were identified in FLEB14Δ3: a 6q− chromosome with the translocated region (q32→qter) of chromosome 14, and a 14q+ chromosome with the translocated region (q15→qter) of chromosome 6. The chromosome region, 14q32, involved in this translocation is known as the locus of the immunoglobulin H-chain gene (17). These results therefore suggested that the immunoglobulin gene rearrangement found in FLEB14Δ3 cells might be due to the chromosomal translocation, not to the normal rearrangement involving the J_H segment, and that the counterpart of the rearrangement might be derived from the sequence in the q15 region of chromosome 6.

Since chromosomal aberrations found in Burkitt's cells are considered to be intimately associated with oncogenesis (24, 25), we tested the tumorigenicity of the FLEB14Δ3 line. Neither the parental FLEB14 nor FLEB14Δ3 showed any tumor formation in athymic mice (0 of 17 and 0 of 26 mice, respectively) after 60 days, consistent with the low plating efficiency (0.7%) of FLEB14Δ3 in soft agar (data not shown) and the absence of the 3A8B marker mentioned above. These results indicate that the FLEB14Δ3 line, although accompanied by a chromosomal abnormality as in Burkitt's cells, has not yet acquired malignancy.

Immunoglobulin gene analysis of FLEB14Δ3. To clarify the rearranged region of the immunoglobulin gene in FLEB14Δ3, Southern blot analyses were performed with the J_H fragment as a probe. While the H-chain gene organization of the parental line FLEB14 was the germ line profile, that of FLEB14Δ3 revealed one rearranged band as well as one germ line band (Fig. 3B). The rearranged J_H fragment was detected when DNA was digested with *Eco*RI or *Kpn*I, which cleaved 3′ to the S_μ region. However, the rearranged J_H fragments were not seen after digestion with *Hin*dIII, which digested 5′ to the S_μ region. These findings suggested that the rearrangement occurred downstream of J_H, but not upstream of the J_H region, where mature B cells are usually rearranged. Combined digestion with *Hin*dIII and either *Eco*RI or *Kpn*I resulted in germ line fragments (data not shown). A Southern blot filter of *Kpn*I digests was first hybridized with the J_H probe and then with the C_μ probe after the first signal was washed off (Fig. 3C). The germ line J_H fragment overlapped with the germ line C_μ fragment (24.5 kb), as expected from the restriction map. However, the size of the rearranged J_H fragment (21 kb) was different from that of the rearranged C_μ fragment (17.5 kb). Other H-chain constant-region genes (C_γ, C_ϵ, and C_α) showed germ line configurations in FLEB14Δ3 cells (data not shown). These results clearly indicate that FLEB14Δ3 cells are rearranged in the area between the J_H and C_μ genes.

TABLE 1. Characteristics of FLEB14 and FLEB14Δ3 cells

Characteristic	FLEB14	FLEB14Δ3
Immunoglobulin		
Surface	−	−
Cytoplasmic	−	−
EBNA	+	+
B-cell antigens		
B4	+ (67%)[a]	+ (80%)
B1	+ (82%)	+ (86%)
B2	+ (31%)	+ (82%)
BB-1	+ (87%)	+ (99%)
3A8B	− (0%)	− (0%)
T-cell antigens		
Leu-1	−	NT[b]
Leu-2a	−	NT
Leu-3a	−	NT
Leu-4	−	NT
CALLA (J5)	2%	0%
Karyotype	46,XY; no translocation	46,XY,t(6;14)
Tumorigenicity	0 of 17 mice	0 of 26 mice

[a] Ratio of antigen-positive cells.
[b] NT, Not tested.

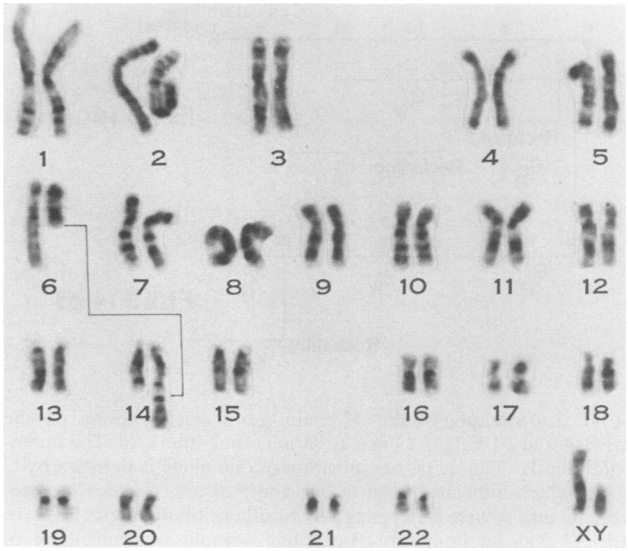

FIG. 2. Representative G-banded karyotype of FLEB14Δ3 cell: 46,XY,t(6;14)(q15;q32). As the result of the reciprocal translocation, FLEB14Δ3 lines had one abnormal chromosome 6 (6q−) and one abnormal chromosome 14 (14q+). The line between abnormal chromosomes 6 and 14 indicates the rearranged site and the reciprocity. Numbers are chromosome numbers.

We also examined the organization of the L-chain genes of FLEB14Δ3 with J_κ and C_λ probes. FLEB14 conserved the germ line configuration of not only the H-chain gene but also the kappa- and lambda-chain genes (19). FLEB14Δ3 also showed the germ line configuration of the J_κ and C_λ genes (Fig. 3D and E). Therefore, we concluded that the rearrangement occurred specifically in one allele of the H-chain gene of FLEB14Δ3 cells.

Molecular cloning of the translocated fragments and their germ line counterparts. To analyze the rearrangement site more directly, we cloned the rearranged fragments from FLEB14Δ3 cells with the J_H segment and 5′-flanking region of the C_μ gene as probes and compared them with the germ line fragment isolated from FLEB14 cells (Fig. 4). The germ line 18.5-kb *Eco*RI J_H fragment from FLEB14 contained the region from upstream of the J_H segment to the 5′-flanking region of the C_μ gene. The rearranged 16.3-kb *Eco*RI J_H fragment of FLEB14Δ3 (designated fragment 6q−) contained the J_H segment and a few kilobases of its downstream segment, which was flanked by an unknown segment at the 3′ side. The rearranged 7.5-kb *Eco*RI 5′C_μ fragment of FLEB14Δ3 (designated fragment 14q+) contained about 2.5 kb of the S_μ region and the 5′-flanking region of the C_μ gene. Upstream of this, another unknown sequence was identified. In the two clones of FLEB14Δ3 the rearrangements seem to have taken place at the similar place close to the S_μ region.

The germ line counterpart of the unknown sequence of FLEB14Δ3 fragment 14q+ was isolated from FLEB14 and its restriction map was constructed. Comparison of the map

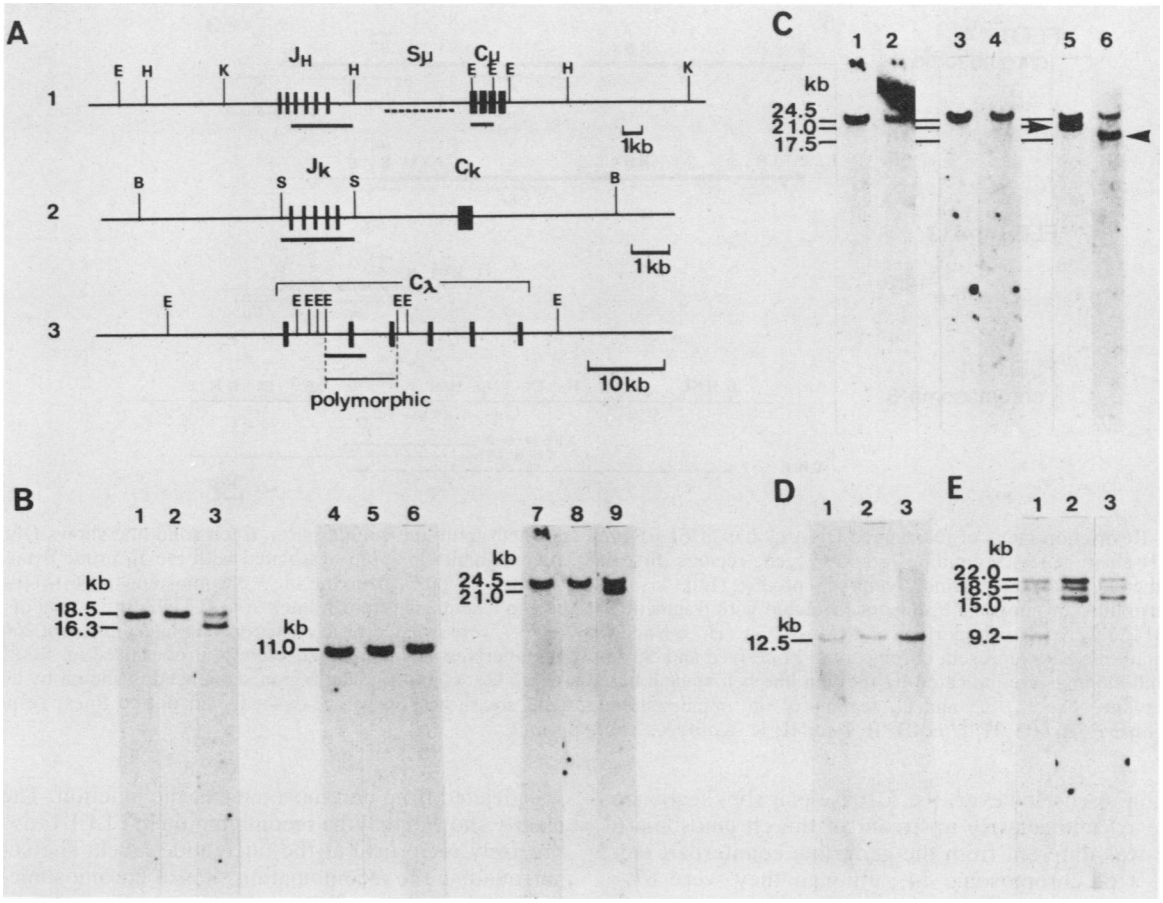

FIG. 3. Southern blot analysis of DNAs of FLEB14Δ3, human placenta, and FLEB14 cells by immunoglobulin gene probes. (A) Restriction maps of human immunoglobulin genes and probes used. Locations of exons are shown by solid rectangles. Lines: 1, H-chain gene; 2, kappa-chain gene; 3, lambda-chain gene. The region of the Cλ genes which contains polymorphism is indicated. Abbreviations: E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; B, *Bam*HI; S, *Sac*I. Probes used are shown by bars below each map. (B) H-chain gene analysis. *Eco*RI (lanes 1, 2, and 3), *Hin*dIII (lanes 4, 5, and 6) or *Kpn*I (lanes 7, 8, and 9) digests of DNAs from human placenta (lanes 1, 4, and 7), FLEB14 (lanes 2, 5, and 8) and FLEB14Δ3 (lanes 3, 6, and 9) cells were analyzed with the J_H probe. (C) Two sequential hybridizations with the J_H and C_μ probes. Filters of *Kpn*I-digested DNAs from human placenta (lanes 1 and 2), FLEB14 (lanes 3 and 4), and FLEB14Δ3 (lanes 5 and 6) cells were first hybridized with the J_H probe (lanes 1, 3, and 5) and then, after washing, with the C_μ probe (lanes 2, 4, and 6). Arrowheads (lanes 5 and 6) indicate the noncomigrating rearranged fragments. (D) Kappa-chain gene analysis. *Bam*HI-digested samples were studied with the J_κ probe. Origin of DNA in each lane: 1, human placenta; 2, FLEB14; 3, FLEB14Δ3. (E) Lambda-chain gene analysis. *Eco*RI-cut samples were studied with the C_λ probe. The additional band found in the human placental DNA may be due to the polymorphism previously described (11). Origin of DNA in each lane: 1, human placenta; 2, FLEB14; 3, FLEB14Δ3.

with those of the rearranged fragments (6q− and 14q+) demonstrated that this germ line region contained the unknown sequences of not only FLEB14Δ3 14q+ but also FLEB14Δ3 6q−. The results were consistent with the assumption that this germ line fragment was derived from chromosome 6 and that the chromosomal translocation in FLEB14Δ3 occurred reciprocally between the S_μ region in the immunoglobulin H-chain gene on chromosome 14q32 and the sequence on chromosome 6q15.

To ascertain that the fragment which was rearranged to the S_μ region originated from chromosome 6, we used mouse × human hybrid MCP-6, which contained only the major portion of human chromosome 6 and the long arm of the human X chromosome in addition to the mouse chromosomes. Southern hybridization with probe x (Fig. 4) detected a single fragment in MCP-6 DNA which was identical to that in FLEB14 and the human placental DNAs (Fig. 5). No bands were identified in the mouse × human hybrid 1W1-5, which contains only the long arm of the human X chromosome. With the same probe, other human B and T cell lines were analyzed, and no rearrangements or polymorphisms have so far been observed (data not shown). In FLEB14Δ3 cell DNA the arrangement of this DNA was observed. At this washing stringency, no hybridizable bands were identified in mouse DNAs (PCC4 and 1RE3). This analysis clearly shows that fragment x is indeed derived from human chromosome 6 and that it is located between 6p21 and 6qter, consistent with the result of the karyotype analysis.

Analysis of sequences surrounding the rearrangement sites. Although the chromosomal and gene mapping data supported reciprocal recombination in many Burkitt's cells, nucleotide sequence determination often revealed that the recombinations were not precisely reciprocal (13, 34). We determined the sequence around the breakpoints of FLEB14Δ3 to see whether the recombination occurred precisely and whether specific sequences were involved in the translocation.

Fragment 6q−, isolated by the J_H probe, had the repetitive sequence (CTGAG or CTGGG) characteristic of the S_μ region (44), followed by the sequence derived from chromo-

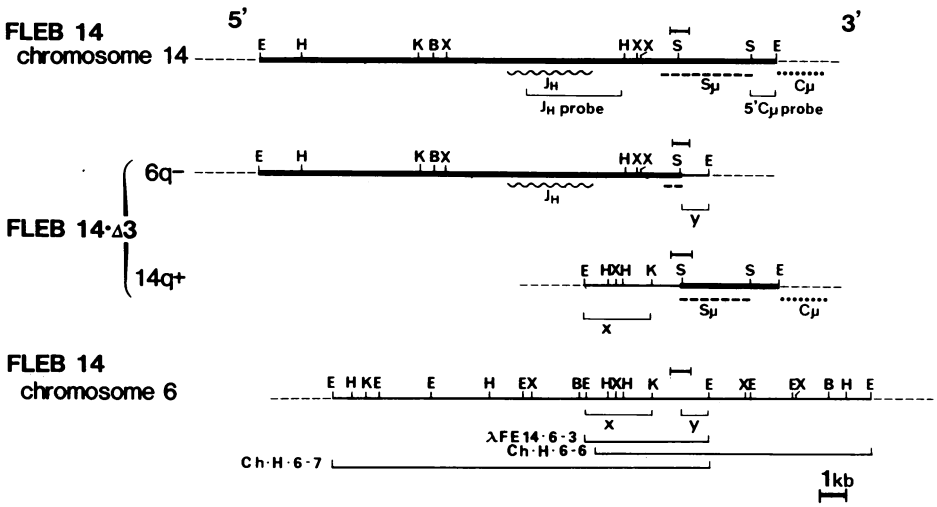

FIG. 4. Restriction maps of rearranged DNA clones of FLEB14Δ3 and their germ line counterparts. Each solid line shows DNA clone; germ line H-chain gene surrounding J_H and C_μ gene regions (first line), recombinant clone (6q−) isolated with the J_H probe from the 6q− chromosome of t(6;14) (second line), reciprocal product (14q+) isolated with the 5'C_μ probe from the 14q+ chromosome of t(6;14) (third line), and the germ line chromosome 6 sequence isolated with fragment x in the 14q+ sequence (fourth line). *Eco*RI DNA fragments of FLEB14 and FLEB14Δ3 were partially purified and cloned. Ch · H · 6-6 and Ch · H · 6-7 were isolated from a Charon 4A phage library of 266BL (37). The thick and thin bars represent chromosome 14-derived and chromosome 6-derived segments, respectively. Probes used for Southern blot analyses and clonings are indicated by the thin line below each bar (J_H, C_μ, 5'C_μ, x, and y). The region sequenced is shown by a bold bar above each line. The J_H, S_μ, and C_μ regions of the immunoglobulin gene are shown by wavy, dashed, and dotted lines, respectively. Abbreviations: E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; K, *Kpn*I; X, *Xba*I; S, *Sac*I.

some 6 (Fig. 6A). However, the 51-base-pair (bp) sequence (sequence A) immediately upstream of the chromosome 6 sequence was different from the germ line counterpart (sequence A') of chromosome 14, although they were 67% homologous. It seems that sequence A' was converted to sequence A in 6q−. The converted sequence A in 6q− was almost identical (92%) to the germ line sequence A located 68 bp upstream of sequence A' (Fig. 6B). On the other hand, fragment 14q+ had the sequence of chromosome 6, which was followed immediately by the S_μ sequence of chromosome 14. It was also clear, however, that the 9-bp sequence was deleted from chromosome 6 at the junction. The results clearly showed that the recombination in FLEB14Δ3 was not precisely reciprocal at the nucleotide level. The sequences surrounding the recombination sites of chromosomes 14 and 6 were not mutually homologous. The chromosomal translocation in FLEB14Δ3 seems to be caused by a nonhomologous recombination with deletion and insertion, which were similar to the events in Burkitt's cells and murine plasmacytoma cells (7, 13, 34). The chi sequence (GCTGGTGG) (23) or its homologs (GCTGG and GCTGA) were frequently found in the S_μ region but not in the 6q15 region.

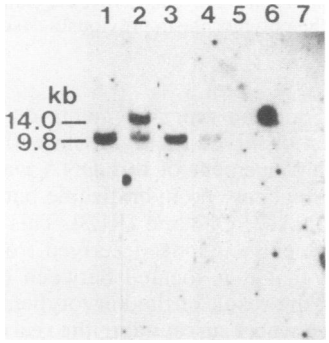

FIG. 5. Localization of the rearranged DNA fragment on chromosome 6. *Bam*HI-digested DNAs on a nitrocellulose filter were hybridized with the ^{32}P-labeled probe x (Fig. 4) under the conditions described in Materials and Methods and washed in 0.1× SSC–0.1% SDS at 65°C. DNA: lane 1, FLEB14; lane 2, FLEB14Δ3; lane 3, human placenta; lane 4, MCP-6 (mouse × human hybrid containing only human translocation chromosome t(6;X)(6p21→6qter::Xq13→Xqter); lane 5, 1W1-5 (mouse × human hybrid containing only human Xq11-Xqter); lane 6, PCC4 (mouse cell line as a control for MCP-6); lane 7, 1RE3 (mouse cell line as a control for 1W1-5). Molecular sizes were determined by using *Hin*dIII-digested phage λ marker DNA.

Sequence surrounding the recombination site of chromosome 6 conserved. Southern blot analysis with probe x from chromosome 6 (Fig. 4) showed a strong 9.8-kb band in *Bam*HI-digested human DNA under low stringency (Fig. 7). Weaker but significant bands were detected in *Bam*HI digests of liver DNAs of rabbit, rat, and mouse, indicating that the human 6q15 sequence was evolutionarily conserved and might be involved in some important cellular function.

DISCUSSION

Translocation in EBV-transformed cells. EBV has been suspected to be the causative agent of Burkitt's lymphoma. However, this has not been proved partly because there are Burkitt's cells with and without EBV genomes and partly because EBV transformants are different from Burkitt's cells containing EBV genomes in many aspects, including cell morphology, cloning efficiency, and tumorigenicity (36). It is worth noting that an EBV-transformed cell line such as FLEB14 was shown to undergo chromosomal translocation in a region similar to that of Burkitt's cells.

EBV transformants generally convey euploidy at least 1 year after establishment, but long-term culture sometimes results in the acquisition of chromosomal abnormalities and malignant features (36, 54). Their chromosomal aberrations, however, show random features such as trisomies or trans-

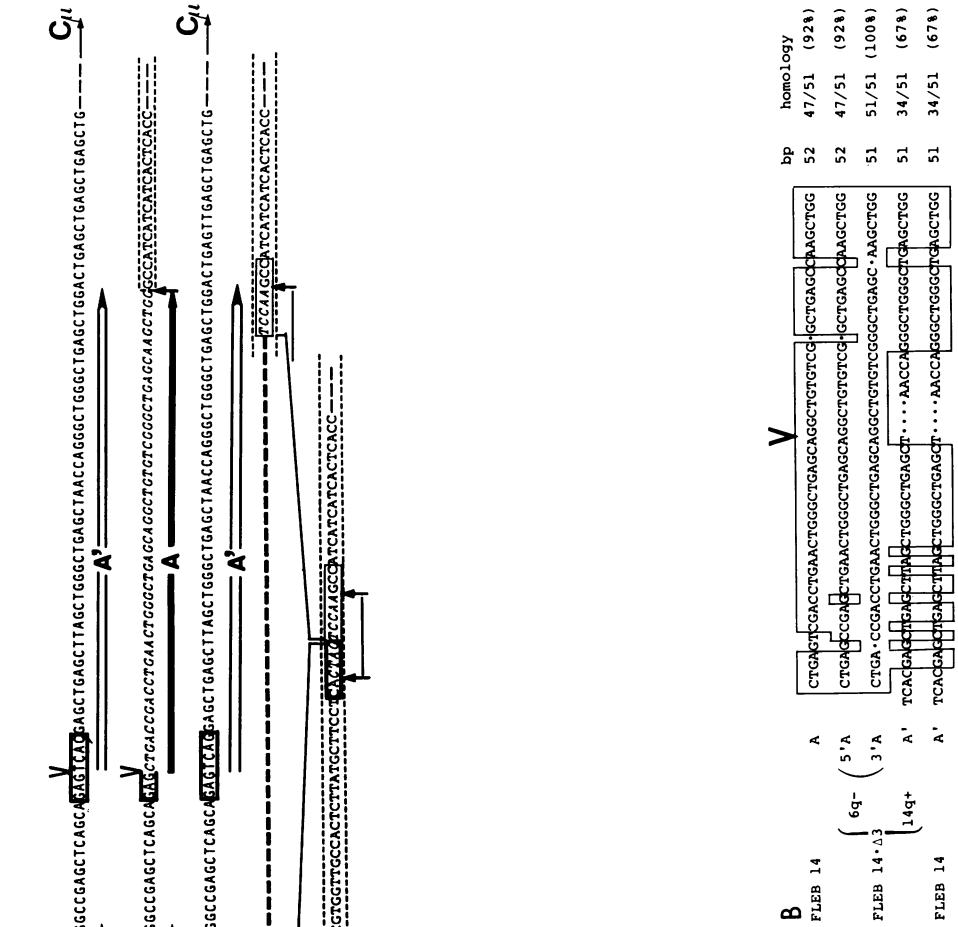

FIG. 6. (A) Nucleotide sequences surrounding recombination sites of t(6;14) translocation and its germ line counterparts. The sequences derived from chromosome 6 (Ch6) and chromosome 14 (Ch14) are shown by dotted boxes and boldface letters, respectively. The break point sequences are aligned so that the chromosome 14-derived sequences match the corresponding normal chromosome 14 sequences. Carets denote the break points of the chromosome 14-derived sequence. Arrows indicate the break points of the chromosome 6-derived sequence. In the germ line chromosome 14 there are two related sequences (67% homology), A and A′. The translocation resulted in the conversion of sequence A′ to sequence A (shown in italics) in fragment 6q− and deletion of the 9-bp sequence (shown in italics) from chromosome 6. The sequence identical to the converted sequence A was also identified in the upstream of the 6q− clone. The sequences related to the recognition sequences of the immunoglobulin gene are indicated in boxes. (B) Comparison of sequences A and A′. The homology was calculated by comparison to the 3′ sequence A of FLEB14Δ3, bases common to which are boxed. The caret indicates the break point in 14q+.

location with low frequency among the population. No uniclonal expansion of EBV transformants with chromosomal translocations including t(8;14) characteristic of Burkitt's cells has been described (18, 27). Recently, one EBV transformant cultured for 16 years was reported to show malignant features with chromosomal aberrations t(6;14)(p12;q32) and t(6;21)(q22;p12) and some others (52). In this case the malignant feature is supposed to be accompanied by the appearance of the chromosome 14 marker, which was probably caused by long-term cultivation, as this marker chromosome was not apparent 1 year after establishment of this line.

On the other hand, FLEB14Δ3 showed clonal expansion at an early stage in the culture and carried the chromosomal aberration in the H-chain gene locus, as do Burkitt's cells. FLEB14Δ3 did not show any sign of malignancy. The translocation in FLEB14Δ3 may therefore have provided a slight growth advantage, leading to the uniclonal expansion of this variant.

Chromosome 14q32 is the locus of the immunoglobulin H-chain gene (17) and is frequently involved in reciprocal translocation with the c-*myc* gene in Burkitt's cells (24, 28, 36). Chromosomal aberrations in this locus are, however, also found in many malignancies other than Burkitt's lymphoma (17, 32). These include malignant lymphoma, B-cell chronic lymphatic leukemia, and prolymphocytic leukemia; all of these cells belong to the B-cell lineage. Molecular analyses of B-cell chronic lymphatic leukemia cells with t(11;14)(q13;q32) (50) and follicular lymphoma cells with t(14;18)(q32;q21) (49) demonstrated that the rearrangements due to chromosomal translocations occurred in the J_H region.

Since rearrangement of the immunoglobulin H-chain gene is the first step in B-cell differentiation (14), it is conceivable that chromosomal aberrations in B-cell malignancies are observed frequently at the locus 14q32, where the immunoglobulin H-chain gene is mapped. Although the FLEB14 line did not show either immunoglobulin production or immunoglobulin gene rearrangement, this line had cell markers characteristic of B cells, indicating that FLEB14 cells had already committed to the B-cell lineage. The chromosomal aberration in FLEB14Δ3 cells suggests that the chromatin structure of the immunoglobulin H-chain gene, especially the S_μ region, was already open for recombination in such immature B cells.

Molecular mechanism of translocation in FLEB14Δ3. The recombination in FLEB14Δ3 was apparently reciprocal but imprecise and nonhomologous. Deletion and conversion of nucleotides were found at the two junctions. The most important observation was conversion of the 51-bp sequence A′ to sequence A in fragment 6q−. It is not clear whether conversion of sequence A′ and recombinations were independent events. The possibility that the two sets of sequence A in addition to sequence A′ were already present in the germ line form due to the polymorphism was excluded by the fact that five J_H clones of FLEB14, including the sequenced one, did not show any difference in the sizes of restriction fragments containing sequence A (data not shown).

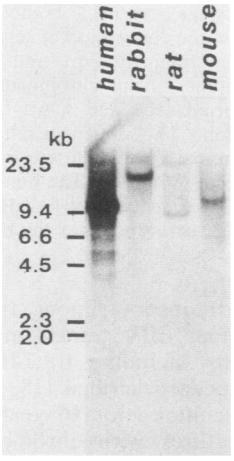

FIG. 7. Evolutionary conservation of translocated gene fragment. DNAs (2 μg) from human placenta, rabbit liver, rat liver, and mouse liver were digested with *Bam*HI, electrophoresed, and blotted to nitrocellulose filters. Hybridization was performed with probe x (Fig. 4). The filter was washed at 50°C with 2× SSC–0.1% SDS and autoradiographed.

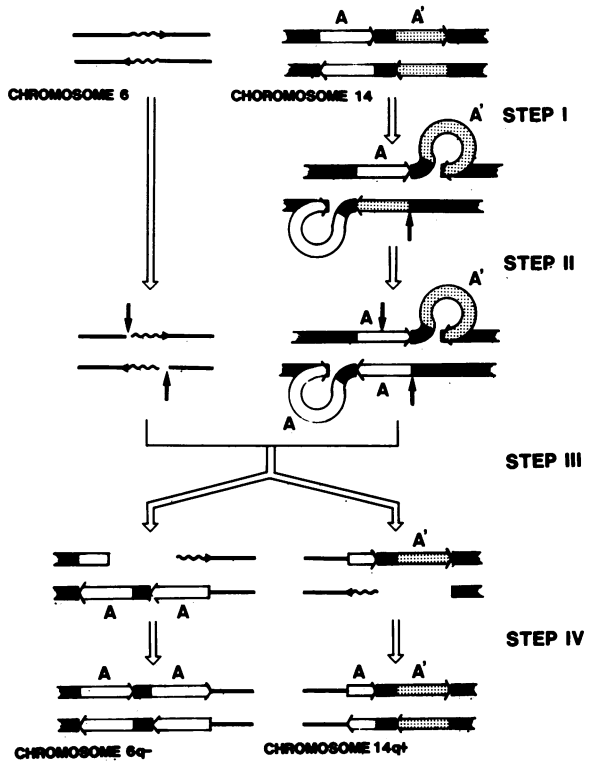

FIG. 8. Model for molecular recombination in FLEB14Δ3. Thin and thick lines indicate complementary strands of chromosomes 6 and 14, respectively. Open and stippled arrows in chromosome 14 show sequences A and A′, respectively (Fig. 5). The wavy lines in chromosome 6 indicate the bases to be deleted after the recombination. The vertical solid arrows indicate nicking sites. Chromosomal translocation between the germ line sequences of chromosomes 6 and 14 (top) results in the marker chromosomes 6q− and 14q+ (bottom). For details, see the text.

To explain the two events by a continuous series of events we propose the model shown in Fig. 8. First, slipping took place between two strands of sequences A and A′ of chromosome 14, resulting in formation of two loop-outs (step I). Nicking and mismatch repair corrected the paired sequence A′ to sequence A (step II). The staggered nicks were introduced within sequence A of the upper strand and at the right end of the corrected sequence A of the lower strand. Nicks were also introduced at the ends of the deleted sequence of chromosome 6. The reciprocal exchange of strands was mediated by ligation between the extruding end of chromosome 14 and the intruding end of chromosome 6 (step III). The gap filling and simultaneous mismatch repairing resulted in duplication of sequence A in fragment 6q− and deletion of the 9-bp sequence from the 6q15 sequence (step IV).

Is recombination in FLEB14Δ3 related to S-S recombination? In mature B-cell malignancies such as Burkitt's lymphoma, the normal V_H-D-J_H and V_L-J_L recombinations had already taken place. Since the S region, especially the S_μ region, is frequently invaded by the c-*myc* gene in Burkitt's cells (for a review, see reference 31), it was suggested that

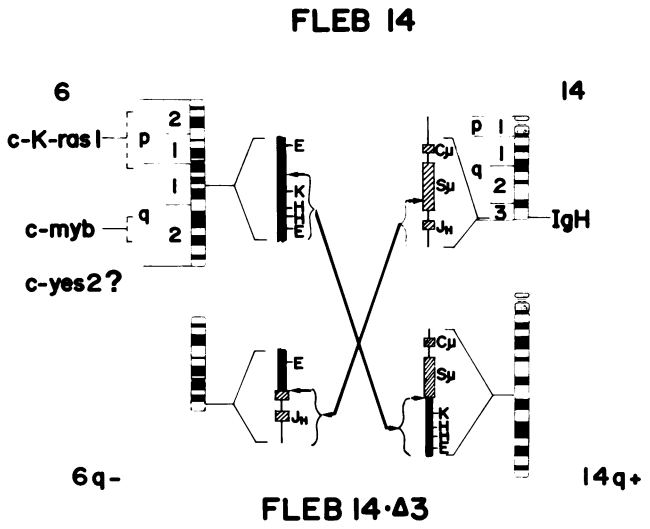

FIG. 9. Summary of the cytogenetic and molecular genetic analyses. Two chromosomes of FLEB14 are shown above, and those of FLEB14Δ3 are shown below. The recombination sites of chromosomes 6 and 14 are enlarged to show the order of identified genes. Locus of immunoglobulin H-chain genes, the proto-oncogenes c-*myb* (6q22-24), c-K-*ras*-1 (6q23-q12), and c-*yes*-2 (locus undetermined) are shown. E, K, and H indicate restriction sites for *Eco*RI, *Kpn*I, and *Hin*dIII, respectively. Horizontal arrows indicate break points.

errors in class switching in mature B cells permitted the invasion of the c-*myc* gene into the S_μ region (7, 48). Sequences of the c-*myc* gene surrounding break points in Burkitt's cells so far published, however, do not necessarily contain the characteristic S region sequences (6, 13, 34). The sequence of chromosome 6q15 in FLEB14 cells was not homologous to the S region sequence either. The chi sequence and its homologs (GCTGG and GCTGA), claimed to be involved in class switching (23), were observed only in the chromosome 14 sequence, but not in the chromosome 6q15 sequence. In addition, less frequent translocation variants t(2;8) and t(8;22), in which the c-*myc* gene recombines with the immunoglobulin kappa and lambda-chain genes, respectively, are also established (30), although these L-chain genes do not carry out class switching.

These results may indicate that the recombination in FLEB14Δ3 is irrelevant to the S-S recombination, assuming that sequence-specific enzymes are involved in the latter. Class switching takes place often in S regions which contain common short repeat sequences (21). Davis et al. (5) proposed that class-specific recombinases might be responsible for S-S recombination. On the other hand, Kataoka et al. (21) assumed that S-S recombination might be a sort of homologous recombination depending on short homology unit sequences. Two recombinant lambda phages which carried different S regions were shown to recombine predominantly within the S regions in *Escherichia coli* extracts (22). The fact that S-S recombination takes place at the preferred regions does not necessarily indicate that S-S recombination is mediated by sequence-specific enzymes. Instead, S region sequences may form unique chromatin structures accessible to many enzymes such as those involved in steps I to IV described above. A recent finding that the C_H regions are transcribed before class switching (46) suggests that these regions form an open chromatin structure. If so, both S-S recombination and chromosomal translocation at the S_μ region may be catalyzed by a recombination system similar to that proposed above which does not recognize any particular sequence per se. It may be the chromatin structure determined by the sequence that decides preferred recombination regions in S-S recombination as well as chromosomal translocation in FLEB14Δ3.

Sequences similar to recognition sequences for V-J recombination. It is interesting that within sequences A and A′ of the germ line form, there are sequences different from the typical S_μ units (GGGCT or GAGCT): GGCTGTGT in sequence A, and GAGTCAC in the 5′ side of sequence A′. GGCTGTGT in particular is found at the break point of fragment 14q+. At the break point of the 6q15 sequence, sequences CACTAG and TCCAAGCC are found. TCCAAGCC is similar to the complementary sequence GGCTGTGT. Sequence GGCTGTGT is also similar to the putative recognition sequence CACTGTG in V_H-D-J_H and V_L-J_L recombinations (14, 34). The sequence (C)ATCTAGT is observed in the break point of the c-*myc* gene in Raji cells with translocation t(8;14), even though the break site is different (6). Sequence CCAAACCC, which is similar to CCAAGCC, is observed near the break point of the c-*myc* gene in two Burkitt's cell lines, BL22 with translocation t(8;14) (34) and BL37 with t(8;22) (13). For translocations in chronic lymphocytic leukemia cells (50) and follicular lymphoma cells (49), involvement of these sequences in recognition of translocation is an attractive hypothesis. However, none of these sequences are conserved among many examples. We therefore prefer the model (Fig. 8) which does not require any specific recognition of sequences. The nicking enzymes might be able to introduce single-stranded breakage at any chromosomal region conformationally open in that particular cell.

No known genes at the break point of chromosome 6. As proto-oncogenes recombined with the immunoglobulin genes in the translocations of Burkitt's cells (1, 4, 25, 29, 48), chronic lymphatic leukemia cells (50), and follicular lymphoma cells (49), we considered the possibility that a proto-oncogene is located at the 6q15 locus. The following proto-oncogenes are mapped on chromosome 6, as shown in Fig. 9: c-*myb* (functional gene, 6q22-24) (9), c-K-*ras*-l (pseudogene, 6p23-q12) (40), and c-*yes*2 (pseudogene, locus undetermined) (43). Using probes of viral counterparts (v-*myb*, v-K-*ras*, and v-*yes*), we did not find any rearrangement in FLEB14Δ3 DNA (data not shown), excluding the involvement of these proto-oncogenes in the FLEB14Δ3 translocation. After submission of this manuscript we learned that human proto-oncogene c-*ros* was mapped in 6q16-q23 (35), which is slightly different from the translocation site in the FLEB14Δ3 line. We are still investigating the possibility that the q15 sequence on chromosome 6 might be a proto-oncogene so far unidentified, as this sequence was evolutionarily conserved.

Studies of clinical cases have revealed that chromosomal aberrations in chromosome 6 are relatively frequently observed in leukemia and lymphoma cells. The hot spots in these cases consisted of two sites: one is locus 6q21-24 and the other is the locus spanning from 6q13 to 6q15 (30). From the above discussion, c-*myb* might be present at 6q21-24, whereas no known oncogenes were identified at 6q13 to 6q15. The 6q15 sequence in FLEB14Δ3 cells might be related to such abnormalities. Since the FLEB14Δ3 line was identified as the uniclonal expansion of cells with chromosomal translocation between the S_μ region and the 6q15 sequence, it is likely that induction or repression of the gene located in 6q15 may endow some growth advantage by this

chromosomal change although the final malignant feature has not been acquired. However, we were unable to detect any expression of the 6q15 sequence in FLEB14 and FLEB14Δ3. The expressed region might be far away from the probe used in the present study.

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