B-Cell Non-Hodgkin’s Lymphoma Cell Line (Karpas 1106) With Complex Translocation Involving 18q21.3 but Lacking BCL2 Rearrangement and Expression


A B-cell non-Hodgkin’s lymphoma (B-NHL) cell line (Karpas 1106) with an unusual three-way translocation involving 18q21.3 has been derived from a patient with mediastinal lymphoblastic B-NHL. Although conventional cytogenetics showed a derivative 18q identical to that seen in cases with t(14;18)(q32.3;q21.3), no translocations of either chromosome 14 could be detected. Instead fluorescent in situ hybridization analysis using a chromosome-18 paint showed that the segment 18q21.3-18qter had become sandwiched on a derivative chromosome X between segments Xqter-c-Xq28 and 13q12-qter, with the centromeric site of 18q21.3 subband juxtaposed to the X sequences. Pulsed-field DNA blots failed to detect rearrangement of the BCL2 gene. Conventional DNA blots using a variety of restriction digests and both 5’ and 3’ BCL2 and FVT1 probes also failed to detect rearrangement in Karpas 1106. A rearranged fragment seen only in HindIII digests with 5’ BCL2 probes may represent a local microaltered, which is either a mutation or small deletion involving the HindIII site as seen in other cases of B-NHL. Neither BCL2 RNA nor BCL2 protein expression were detected. These and other data suggest that genes at 18q21.3, other than BCL2 and FVT1, may be targets for translocation in certain subgroups of B-NHL.

CHROMOSOMAL translocation t(14;18)(q32.3;q21.3) was first identified in follicular B-cell non-Hodgkin’s lymphoma (B-NHL) in 1976 by Fukuhara and colleagues.1 Identical translocations were subsequently detected in about 30% of diffuse B-NHL2 and rarely in acute leukemias of some 14 could be detected. Instead fluorescent in situ hybridization analysis using a chromosome-18 paint showed that the segment 18q21.3-18qter had become sandwiched on a derivative chromosome X between segments Xqter-c-Xq28 and 13q12-qter, with the centromeric site of 18q21.3 subband juxtaposed to the X sequences. Pulsed-field DNA blots failed to detect rearrangement of the BCL2 gene. Conventional DNA blots using a variety of restriction digests and both 5’ and 3’ BCL2 and FVT1 probes also failed to detect rearrangement in Karpas 1106. A rearranged fragment seen only in HindIII digests with 5’ BCL2 probes may represent a local microalteration, which is either a mutation or small deletion involving the HindIII site as seen in other cases of B-NHL. Neither BCL2 RNA nor BCL2 protein expression were detected. These and other data suggest that genes at 18q21.3, other than BCL2 and FVT1, may be targets for translocation in certain subgroups of B-NHL.

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MATERIALS AND METHODS

Patient Material, Cell Culture, and Cytogenetics

The cell line was derived from a 23-year-old lady with no significant previous medical history who presented in September 1983 with mediastinal lymphoblastic B-NHL. She was treated with intravenous combination chemotherapy (cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone) and initially remitted, but relapsed in May 1984 with widespread disease. Despite further therapeutic attempts, she died 4 months later with progressive chemotherapy-resistant B-NHL including massive ascites, bilateral pleural effusions, and CNS disease. Cell lines were established from
B-CELL LINE WITH 18q21.3 LACKING BCL2

Fig 1. Schema of both 5' and 3' regions of the BCL2 and FVT genes involved in chromosomal translocations in B-cell malignancies and position of DNA probes used in this study. The 5' and 3' BCL2 regions are separated by an intron of ~250 kb. E denotes EcoRI sites; H, HindIII sites; B, BamHI sites. H* represents HindIII site lost on one BCL2 allele in Karpas 1106. The map linking mbr and mc has been taken with permission from Kiem et al.22

some preparations were made after the cells were treated with BrdU (20 mg/mL) for 17 hours followed by ethidium bromide/colcemid incubation for the last 90 minutes before harvest. Before denaturing, chromosome preparations were treated in 2× saline sodium citrate (SSC) for 60 minutes at 37°C.

Chromosomal DNA was denatured in 70% formamide/2× SSC at 75°C for 2 to 4 minutes, then dehydrated. Probe (paint) DNA plus unlabeled Cot-1 DNA (5 mg/mL; Gibco BRL, Gaithersburg, MD) were denatured at 75°C for 10 minutes, allowed to preanneal for 90 minutes at 37°C and laid onto prewarmed slides under sealed coverslips. The hybridization was performed at 42°C for 12 to 15 hours. After hybridization, the slides were washed three times on 2× SSC, 50% formamide at 42°C followed by two washes in 2× SSC and once on 0.1× SSC at 42°C. Visualization of the hybridized probe was achieved after incubation with (1) 5 mg/mL fluorescein isothiocyanate (FITC)-avidin DCS, (Vector Laboratories, Burlingame, CA), (2) 5 mg/mL biotinylated goat antiavidin (Vector) and (3) FITC-avidin DCS. All incubations were at 37°C for 30 minutes. Three washes, each 3 minutes in length, were performed between layers using 4× SSC, 0.05% Tween 20. Chromosomes were counterstained with DAPI dissolved in antifading solution (Citifluor, Canterbury, UK) at a final concentration of 80 mg/mL. Images were captured with CCD Camera (Photometrics, Tucson, AZ) aided by dedicated software (SmartCapture, Digital Scientific, Cambridge, UK). The G-banded appearance of the counterstained chromosomes was obtained by image processing using the same program.

**Immunophenotyping**

Cell surface antigen expression was detected using monoclonal antibodies (MoAbs) and flow cytometry as described.20 Cytoplasmic BCL2 expression was assessed using a cocktail of BCL2 MoAbs21 and both immunocytochemistry and flow cytometry after cell permeabilization.22,23 Cell lines Karpas 422 and DoHH2, which both exhibit a BCL2 mbr-IgJ fusion gene were used as positive controls.24,25 As a negative control, the primary antibody was omitted.
B-CELL LINE WITH 18q21.3 LACKING BCL2

Table 1. Immunophenotype of B-NHL Cell Line, Karpas 1106

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Expression</th>
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<tbody>
<tr>
<td>CD5</td>
<td>−</td>
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<td>CD10</td>
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<tr>
<td>CD19</td>
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<tr>
<td>Igλ</td>
<td>−</td>
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<tr>
<td>BCL2</td>
<td>−</td>
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</table>

Immunophenotypic analysis was performed by flow cytometry for both cell surface and cytoplasmic BCL2 antigen.

Abbreviations: +, weak positive expression; ++, strong expression; −, no detectable expression.

DNA and RNA Studies

Conventional DNA blotting. Conventional DNA blotting was performed as described.26 High molecular-weight DNA from Karpas 1106 and normal peripheral blood mononuclear cells was digested to completion with the following restriction endonucleases: EcoRI, HindIII, BamHI, PstI, BglII, EcoRV, and KpnI (Promega, Madison WI).

Pulsed-field DNA blots (PFGE). Pulsed-field DNA blots were performed as described.27 DNA blocks were digested with restriction endonuclease NotI which yields a 650-kb germ line BCL2 fragment.26

RNA studies. Northern blots were performed using poly (A+) mRNA as described.19 Quantitation of the amount of mRNA applied and assessment of mRNA integrity were assessed by probing with a glyceraldehyde-3-phosphate dehydrogenase cDNA probe.

DNA probes. All DNA probes were used as gel-purified inserts and were labeled with 32P-deoxycytidine triphosphate (dCTP) to a specific activity of ≈ 2 x 106 disintegrations per minute (dpm)/µg DNA by the method of oligopriming. Derivation of the probes used in this study may be found in the following references: FVTI: probes for the FVTI locus were 140 CA, 76/1, and 35R6. Probe 140 CA is a 0.4-kb KpnI FVTI 3' cDNA fragment, whereas 76/1 and 35R6 are genomic clones lying immediately 5' and 30 to 40 kb 5' of the FVTI gene, respectively. 76/1 is a 0.8-kb EcoRI-XbaI fragment and 35R6 a 0.8-kb HindIII fragment13 (and R. Rimoh, personal communication, November 1993); 5' BCL2: two genomic clones lying 5' of the BCL2 coding sequences were used: pHb 0.6, a 0.6-kb HindIII-BamHI fragment27 and pXk19.1, a 1.9-kb XhoI fragment.26 Additionally, a 1.6-kb cDNA clone, pB16, encoding part of the first and second exons of BCL2, was also used26; 3' BCL2 (mbr): clone pFL-1, a genomic fragment spanning the mbr; 3' BCL2 (mcr): clone pFL-2, a genomic fragment spanning the minor cluster region13; 3' BCL2 (mbr-mcr): clone pMW-1, a 1.3-kb EcoRI-HindIII genomic fragment lying between the mbr and mcr.27 The relationship of these FVTI and BCL2 probes is shown in Fig 1. References for the derivation of immunoglobulin (Ig) probes may be found in reference 26.

RESULTS

Cytogenetic and FISH Studies

A mainline cell clone was seen in all analyzed cells of Karpas 1106P, which was found to have the following chromosomal constitution (Fig 2): 49,X,del(2)(p11.2p13.3), der (3)(t;2;3)(p13.3;p25.1), +19(p), ins(12;?) (q13.1q13.3), del(14)(q11.2q13.1), del(15)(q11.2q15.3), der(18) t (X;13;18)(q28;q21.3;q12.1), −20, del(20)(q13.1q13.3) x 2, der(X)t(X;13;18) (q28;q21.3;q12.1), +i(Xp).

To confirm this, dual-color FISH analysis with a chromosome 18 (in green) and X (in red) paints was performed (Fig 3). This showed that the 18q21.3-qter segment Whas retained in the genome as part of the der(X) marker, sandwiched between segments Xqter-c-Xq28 and 13q12.1-qter (Fig 3D, inset). The presence of material from chromosome X at the telomeric end of der(18) marker (see Fig 3A) is suggestive of a two-step mechanism of marker formation. Firstly t(X;18) (q28;q21.3) followed by rearrangement between the der(X) and 13. This interpretation is in agreement with the results of the dual-painting experiments with chromosome 18 and 13 (Fig 3B) as well as X and 13 (Fig 3C).

Chromosomes 14, 15, and 20 were found to have a partially deleted long arm. FISH analysis with relevant chromosome paints showed the presence of only two signals, thus suggesting that these deletions were interstitial. Painting with chromosome 12 probe showed uniform signal on the der(12) chromosome, and this finding suggests that the inserted material belongs to chromosome 12. The t(2;3) was also confirmed with dual-color FISH chromosome painting.

Immunophenotypic Analysis

Both Karpas 1106A and 1106P had the phenotype of mature, class-switched IgG expressing B-cells as shown in Table 1. There were no differences between the 1106A and P sublines. BCL2 expression was sought on three separate occasions in two laboratories by both flow cytometry and immunocytochemistry; no BCL2 expression was detected under conditions when both Karpas 422 and DoHH2 exhibited strong staining (Fig 4).

DNA and RNA Studies

Rearrangements of both BCL2 and FVTI were sought in Karpas 1106. Results are summarized in Table 2. In pulsed-field blocks digested with NotI, no BCL2 rearrangements could be detected using 3' BCL2 probe pMW1 (data not shown). To confirm these data, and to extend the area analyzed, rearrangements in Karpas 1106 were sought using both BCL2 and FVTI probes in a wide variety of restriction digests by conventional DNA blot. No rearrangements were detected using 3' BCL2 and FVTI probes. Additionally, no rearrangements were detected using the 5' BCL2 probes except in HindIII digests (Fig 5). The size of the rearranged HindIII fragment is consistent with a local microalteration at the 5' end of the BCL2 gene of one allele in the vicinity of the 5' HindIII site (Fig 1) resulting in the loss of that HindIII site. Furthermore, these data indicate that the cytogenetically observed breakpoint at 18q21.3 fell outside both BCL2 and FVTI loci.

Studies with probes from the Ig loci showed that both Karpas 1106A and 1106P sublines had identical biallelic IgH rearrangements, indicative of a common clonal origin. Both alleles of Igκ were deleted, with biallelic Igκ re-
Flow cytometric profiles of B-NHL cell lines Karpas 1106 and DoHH2 stained with a cocktail of BCL2 MoAbs after permeabilization of the cell membrane. Abscissa, fluorescent intensity; Ordinate, log cell number. DoHH2 shows level of staining comparable with that seen in other B-cell lines with t(14;18)(q32.3;21.3), whereas only background staining was seen in Karpas 1106. Identical results were obtained reproducibly in two of our laboratories and were confirmed by immunocytochemistry.

Fig 4. Flow cytometric profiles of B-NHL cell lines Karpas 1106 and DoHH2 stained with a cocktail of BCL2 MoAbs after permeabilization of the cell membrane. Abscissa, fluorescent intensity; Ordinate, log cell number. DoHH2 shows level of staining comparable with that seen in other B-cell lines with t(14;18)(q32.3;21.3), whereas only background staining was seen in Karpas 1106. Identical results were obtained reproducibly in two of our laboratories and were confirmed by immunocytochemistry.

No BCL2 RNA expression could be detected in Northern blots using 2 µg of poly(A⁺) RNA (Fig 6).

DISCUSSION

The cell line Karpas 1106 is of interest for two reasons. Firstly, although it was thought initially that BCL2 expression was a specific marker of the t(14;18)(q32.3;q21.3) translocation, it has become clear that expression of this gene is, in fact, widespread, particularly in tissues characterized by programmed cell death or apoptosis. Most follicular and diffuse B-NHL and B-CLL express abundant BCL2 irrespective of whether they carry the t(14;18)(q32.3;q21.3) or not.22,33,37 Also, levels of BCL2 comparable with those seen in B-NHL with t(14;18)(q32.3;q21.3) may be seen in the breast carcinoma cell line MCF-7 with no detectable genomic alteration of the BCL2 gene.38,39 Therefore, it is surprising that the Karpas 1106 cell line should lack expression of BCL2. To our knowledge, the only other B-NHL cell line with an 18q21.3 translocation that fails to express BCL2 is

<table>
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<th>Table 2. Genotype of B-NHL Cell Line, Karpas 1106</th>
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Karpas 1106A and 1106P showed identical biallelic JH rearrangements. Otherwise, both Cγ and Cκ were biallelically deleted consistent with the observed IgG expression. No rearrangement of BCL2 was seen by PFGE in a Not I digest when probed with pMW1. Abbreviations: G, germline configuration; R, rearranged configuration; D, deleted DNA sequences. *Rearrangement of 5′ BCL2 seen in HindIII digests only, which was considered to represent a local microalteration (see text).
**B-CELL LINE WITH 18q21.3 LACKING BCL2**

**PROBE: BCL2 (pFL-1)**

Track: A B

< 9.5kb < 7.5
< 4.4 < 1.3

**PROBE: GAPDH**

Fig 6. Northern blot of Karpas 1106 RNA. Poly (A)+ RNA from Karpas 422 (track A) and Karpas 1106 (track B) cell lines was blotted and probed with BCL2 probe pFL-1 (upper panel) and a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (lower panel). No BCL2 mRNA expression was detected in Karpas 1106.

SU-DUL5, in which one allele of BCL2 has been disrupted in a three-way translocation with MYC and IgJμ. Other B-NHL lines that do not express BCL2 (eg, SU-DHL9) do not exhibit 18q21.3 translocations. Our examination of other B-NHL lines that have 18q21.3 translocations but lack BCL2 rearrangement on DNA blot has shown abundant BCL2 expression in all three cases (M.J.S. Dyer, unpublished observations, December 1993). In Karpas 1106, it is possible that microalterations (either mutations or small deletions) at the 5' end of the BCL2 gene (as detected by the rearrangement seen in HindIII digests alone) may have inactivated the promoter of one allele, whereas the other promoter may be physiologically repressed. Such microalterations in the 5' region of the BCL2 gene have been previously reported both in B-NHL lines and in fresh patient material. In one case of follicular B-NHL, it was shown that these alterations reflect small deletions of the 5' region of BCL2. Similar deletions have been observed in the B-NHL cell line SU-DHL5; these deletions involved the major sites of initiation of BCL2 transcription.

Secondly, despite lacking BCL2 expression, Karpas 1106 nevertheless has a translocation involving 18q21.3, although this does not appear to involve the BCL2 gene directly. These and other data suggest that genes at 18q21.3, other than BCL2, may have a role in the pathogenesis of certain subsets of B-NHL. Therefore, the Karpas 1106 cell line may allow the study of mechanisms of B-cell neoplasia independent of BCL2 expression.

**ACKNOWLEDGMENT**

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B-cell non-Hodgkin's lymphoma cell line (Karpas 1106) with complex translocation involving 18q21.3 but lacking BCL2 rearrangement and expression

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