RESEARCH ARTICLE

Synthesis of Kappa Light Chains by Cell Lines Containing an 8;22 Chromosomal Translocation Derived from a Male Homosexual with Burkitt's Lymphoma

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Abstract. *Three cell lines were derived from a homosexual patient with probable acquired immunodeficiency syndrome and Burkitt's lymphoma. The cell lines produce an unusual strain of Epstein-Barr virus which will both transform cord blood lymphocytes and induce early antigens in Raji cells. Translocations between chromosomes 8 and 22 have occurred in all three lines, but the cells synthesize immunoglobulin M with light chains of the κ type, in contrast to the usual concordance between a translocation involving chromosome 22 and λ chain synthesis. Both κ genes and one λ gene are rearranged. These findings indicate either that translocation may occur as a separate event from immunoglobulin gene rearrangement or that the proposed hierarchical sequence of immunoglobulin gene rearrangements is not always adhered to. The data also imply that in cells containing a translocation between the long arm of chromosome 8 and a chromosome bearing an immunoglobulin gene, alteration of cellular* myc *expression may occur regardless of the immunoglobulin gene that is expressed.*

Burkitt's lymphoma is a monoclonal malignant proliferation of lymphoid cells that nearly always express surface immunoglobulin of the immunoglobulin M (IgM) class (*1*). The cells also contain a reciprocal chromosomal translocation involving the long arm of chromosome 8 (q24 → qter), the location of the c-*myc* oncogene (*2*), and one of the chromosomes containing the immunoglobulin genes, namely, 14 (heavy chains), 22 (λ light chains), or 2 (κ light chains) (*3–6*). Most of the Burkitt tumors occurring in Africa contain Epstein-Barr virus (EBV) DNA, whereas most of those occurring in Europe or the United States lack the EBV genome (*7–9*). Other differences between African and American Burkitt lymphomas have been described, based largely on studies of continuous cell lines derived from the tumors (*10–12*). Recently, undifferentiated lymphomas resembling or identical to Burkitt's lymphoma have been described in homosexual individuals with the acquired immunodeficiency syndrome (AIDS) (*13*). Although the number of reported tumors in patients with AIDS is still very small, the majority have contained EBV DNA. Few of these tumors have been studied cytogenetically, but recently the results of karyotyping in two "Burkitt-like" lymphomas in homosexual men with AIDS were reported. The cells of one had an 8;14 translocation (q24;q32) and the cells of the other an 8;22 translocation (q24;q11) (*14*).

We describe here the characteristics of three cell lines derived from a Burkitt lymphoma in a Norwegian patient with features of the AIDS-related disease complex. The cell lines produce an EBV strain with unusual biological properties. They also contain an 8;22 chromosomal translocation and synthesize IgM of κ type. This differs from the more usual relation between light chain expression and chromosomal translocation described by Lenoir and colleagues in which cell lines or tumors with an 8;22 translocation express λ, while those with a 2;8 translocation express κ light chains (*15*). Our finding raises a number of questions concerning the proposed hierarchical sequence of light chain rearrangements as well as the interpretation of the significance of the chromosomal translocations that occur in Burkitt's lymphoma.

The patient. The patient was a 30-year-old homosexual male with a 4-month history of lymphadenopathy, fatigue, and weight loss. He presented with widespread Burkitt's lymphoma, confirmed histologically (Fig. 1) and cytologically, involving the bone marrow, peripheral blood, liver, spleen, peripheral lymph nodes, pleurae, and central nervous system. Ulcerating lesions positive for herpes simplex virus were seen on the buttocks. Tests for hepatitis B core and surface antigens were positive and elevated antibody titers to EBV (antibody to viral capsid antigen, 1:1280; antibody to early antigen, 1:80; antibody to EBV nuclear antigen, 1:20) and cytomegalovirus (CMV; immunoglobulin G, 1:3200) were present. There was an inverted ratio between T4 and T8 peripheral T lymphocytes (0.4, whereas the normal is > 1.6). After an initial good response to chemotherapy, the patient developed an acute widespread demyelinating syndrome possibly associated with intrathecal therapy, and died from a cerebral hemorrhage which occurred after the removal of a cerebral ventricular shunt placed because of raised intracranial pressure.

Derivation of cell lines. Before the patient received therapy, 2 ml of heparinized bone marrow were obtained and centrifuged through Ficoll/Hypaque. Interphase cells were washed twice in RPMI 1640 medium and cultured in RPMI 1640 with 20 percent fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) in an atmosphere of 5 percent CO_2. Cells from the peripheral blood were similarly processed while cells from the pleural effusion were diluted to a concentration of 1 million per milliliter in the same tissue culture medium. All cells were cultured in a 5 percent CO_2 atmosphere. In all cases there was continuous cell growth and cells were subcultured by diluting 1:5 with fresh complete tissue culture medium, as

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above, every 3 to 4 days. The cell lines were designated PA682, PEl being derived from the pleural effusion, BMl from bone marrow, and PB from peripheral blood.

Surface characteristics and immunoglobulin secretion. The three cell lines were tested by an indirect immunofluorescent technique for the expression of a number of B-cell antigens including surface IgM (μ heavy chain), κ- and λ-light chain, the Bl and B2 antigens (*16*, *17*), and the common acute lymphoblastic leukemia antigen (CALLA) (*18*). In addition, the expression of HLA antigens including β2-microglobulin, class I (HLA ABC) and class II (HLA DR) antigens was examined (*19*).

Immunofluorescence on the cells was assessed by microscopy (Leitz-Orthoplan) and quantitative measurements of fluorescence intensity distributions for each antigen were performed with a cytofluorimeter (FACS IV, Becton and Dickinson). Each antibody was used at least three times on separate occasions for each of the cell lines, which were in exponential growth phase at the time of the measurements, and μ, κ, and λ were each assessed by two different monoclonal antibodies (*19*).

Like the original tumor cells, all three cell lines expressed surface IgM of κ light chain type exclusively. The level of fluorescence amounted to approximately a quarter of the IgM level of Daudi cells. The PB line appeared to express less μ chain but the same level of κ light chain as the other two lines. Expression of λ light chain was consistently negative in all three lines tested with the monoclonal antibodies. The absence of λ light chain expression was further confirmed by use of the $F(ab')_2$ fragment of a heterologous antiserum to human λ light chain that was raised in rabbits and coupled with fluorescein isothiocyanate (Cappel, Westchester, Pennsylvania). Immunofluorescence profiles for κ and λ light chain expression are shown in Fig. 2, in which the PA682 PE1 line is compared with a Burkitt lymphoma cell line (MC 116) that is positive for λ light chain (*10*). The fluorescence distribution curves for PE1 are representative of results obtained with all three lines. All three cell lines showed a clear but low positivity for B2 and CALLA and they were strongly positive for Bl as well as for β2-microglobulin (see Table 1) and class I and II HLA antigens. Complement receptors, detected by the binding of complement-coated fluorescent bacteria, were present on 10 to 20 percent of the cells.

Immunoglobulin secretion was demonstrated by assaying culture supernatants by an ELISA technique (*20*). The light chain type was shown to be exclusively κ in several experiments in which we used microwell plates coated with several different antibodies to κ chains and detected light chains with alkaline phosphatase-coupled antibodies. Only when both the coating and detecting antibodies were directed against κ was marked reactivity observed. With all other antibody combinations reactivity was not above background. Approximately 1 μg of IgM per milliliter per 24 hours (measured by ELISA, using antibodies specific for μ chains) was secreted by the PE1 and BM1 cell lines in cultures initiated at 5×10^5 cells per milliliter. The PB cell line secreted 40 percent of this amount. These results were consistent with the amount of κ light chain detected in supernatants by ELISA assays directed against κ. The characteristics of the cell lines are summarized in Table 1.

Epstein-Barr virus association. All three cells lines contained the EBV nuclear antigen (EBNA) demonstrated by indirect anticomplementary immunofluorescence, and also expressed the viral capsid antigen in 10 to 20 percent of aged (14 days at 35°C) cells.

Electron microscopy revealed the presence of intranuclear herpes virions (Fig. 1). The presence of EBV DNA has been confirmed by Southern blotting analysis of purified cellular DNA subjected to digestion with the restriction enzyme Eco RI, and probed with ^{32}P-labeled purified EBV DNA (data not shown). The presence of EBV DNA was also detected in the original tumor cells both by immunofluorescent detection of EBNA, and by DNA-DNA reassociation kinetics which indicated an average EBV

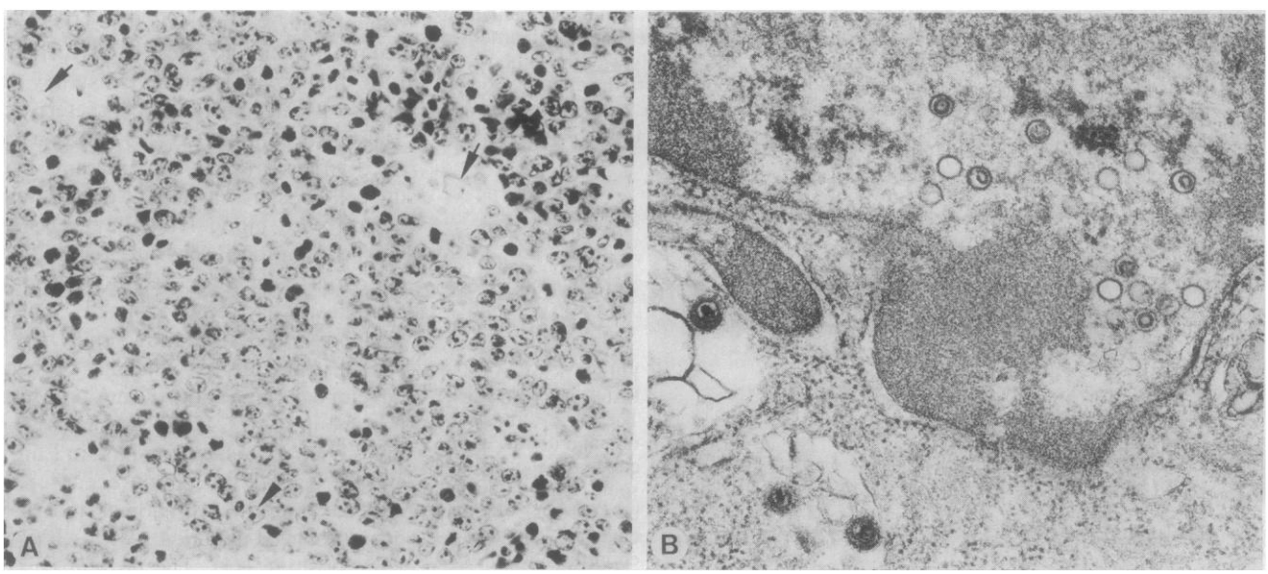

Fig. 1. (A) Light micrograph of original tumor in lymph node. The normal lymphocyte population was diffusely replaced by this blastic, uniform tumor cell population. Tumor cell nuclei and "starry sky" macrophage nuclei (arrows) are similar in size. Multiple small nucleoli are present in virtually all tumor cells. Rare single prominent nucleoli (arrow head) and nuclear pleomorphism are present, but most of the cells are typical of Burkitt's lymphoma (hematoxylin and eosin, ×500). (B) Electron micrograph of cells of the PA682 BM1 line. The cells were prepared by fixation for 1 hour in 1.25 percent glutaraldehyde in 1*M* phosphate buffer. They were then embedded in epoxy resin, sectioned, and stained with uranyl acetate and lead citrate. Intranuclear capsids consistent with herpesvirus particles, half with DNA cores, are apparent. Enveloped virions (lower left) were also found at the cell surface and admixed with cell debris. No cytoplasmic virions or dense bodies typical of CMV were found. These inclusions are therefore interpreted as EB virus (×60,000).

DNA content of 40 genome-equivalents per cell. All the PA682 lines initially produced virus in high quantity. This virus was capable of both transforming cord blood lymphocytes and inducing early antigen in Raji cells—a phenomenon only recently described in some P3HRl cell lines (*21*). The techniques for these assays have been described (*21*).

Karyotyping. All cells contained an 8;22 translocation (Fig. 3) identical to that seen in the fresh tumor cells. The modal chromosome number was 46; G-banding analysis showed that the cells had a karyotype of 46, XY, t(8;22) (q24;q11-12). Many of the cells also expressed a duplication or double duplication of chromosome 1 (q21-32). Identical results were obtained with fresh tumor cells (*22*).

Structural arrangements of immunoglobulin and c-myc genes. Southern blotting analysis of cellular DNA from the PA682 PE and BM lines digested with Bam HI and probed with cloned human constant region μ (C_μ) DNA or constant region κ (C_κ) DNA demonstrated a single rearranged μ gene (Fig. 4A) and two rearranged κ genes (Fig. 4B). While Bam HI digests did not allow an assessment of λ gene rearrangements to be made, we have observed rearrangement of one λ allele following Eco RI and Hind III DNA digestion (Fig. 5). At present, however, we cannot completely exclude the possibility that a λ gene polymorphism accounts for this finding, although the fragment pattern is not consistent with recognized polymorphisms (*23*).

Southern blotting analysis of Eco RI and Bam HI digests of PA682 PEl and BMI DNA alongside PAF SV40-transformed human cellular DNA, P3HR1 DNA (Burkitt's lymphoma, 8;14 translocation), and BL2 DNA (Burkitt's lymphoma, 8;22 translocation) did not show rearrangement of the c-*myc* gene in any of these cell lines (Fig. 6). Previously, a rearrangement of c-*myc* in P3HRI DNA was observed with Eco RI digests although not with Bam HI or Hind III digests (*24*). Such rearrangement of c-*myc* in P3HRl has been detected only in a late passage of P3HRl, and was not observed in the original P3HRl cell line or its hybrids studied in this laboratory (*25*).

At present we have not ascertained whether the c-*myc* gene remains on chromosome 8 or is translocated into chromosome 22 in the PA682 cell lines. However, the gene itself and its flanking sequences appear intact since electrophoresis of PA682 DNA after digestion with Bam HI or Eco RI, both of which enzymes cut outside the c-*myc* gene, shows that c-*myc* is in its germ line configuration.

Discussions and conclusions. These cell lines derived from a homosexual patient with stigmata of the AIDS syndrome are unique in several respects. Like the original tumor cells they express B-cell surface characteristics. They also contain EBV DNA and produce virus particles. Supernatants from the cell lines are capable of transforming human cord blood lymphocytes as are most strains of EBV, but, remarkably, the virus from these cell lines also induced early antigen in Raji cells, a property possessed until now only by the P3HRl strain of EBV which has probably developed a mutation during prolonged passage in vitro (*21*). EBV-positive Burkitt's lymphoma is uncommon in Europe and the United States, but recent reports indicate that the lymphomas that have been observed in patients with AIDS are EBV positive (*13*). The present case is consistent with this finding and it will be important to determine whether non-African patients with EBV-positive tumors, as well as patients with AIDS, consistently carry the unusual EBV strain observed here.

Until recently, only incomplete karyotyping was available on undifferentiated

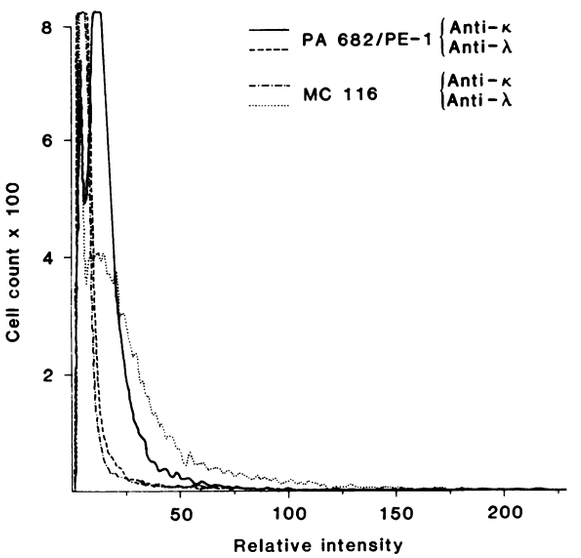

Fig. 2. Fluorescence intensity distribution of κ and λ immunoglobulin light chain on the cell line PA682 PE1 compared with the λ light chain–positive cell line MC 116. The histograms shown are flow cytometric measurements (FACS IV, Becton and Dickinson) of the cell lines PA682 PE1 and MC 116. Each cell line was stained for κ light chain and λ light chain expression. Cells were incubated for 45 minutes with a 1/100 dilution of a mouse monoclonal antibody to human κ immunoglobulin light chain (Becton and Dickinson) washed three times in phosphate-buffered saline (PBS), and incubated for a further 45 minutes in a 1:40 dilution of an FITC-coupled goat antibody to mouse IgG (Cappel). To test the expression of λ light chain, we incubated cells for 45 minutes in a 1:20 dilution of an FITC-coupled $F(ab')_2$ fragment of goat antibody to human λ immunoglobulin light chain (Cappel). The cell line PA682 PE1 shows a definite but low expression of κ light chain whereas the profile of MC 116 for κ is identical to a control incubated only with the antibody to mouse IgG (not shown). PA682 PE1 showed a clearly negative profile with the anti λ antibody. MC 116 served as a positive control for surface λ light chain expression. Similar profiles were obtained with monoclonal or heterologous antibodies against κ and λ [see text and (*19*) for details] for all three cell lines PA682 PE1, BM1, and PB.

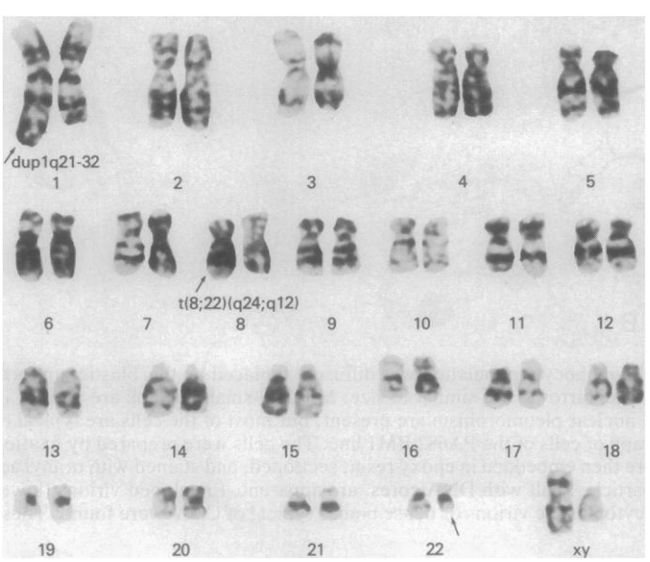

Fig. 3. Karyotype of PA682 PE1 cell line. The 8;22 translocation and double duplication of chromosome 1 are indicated by arrows. Similar changes were detected in the other PA682 cell lines. For the techniques of banding and interpretation of metaphases, see (*22*).

lymphomas in homosexual males, and the translocations associated with Burkitt's lymphoma were not detected (*13*). However, we and others have observed characteristic translocations in these tumors (*14*, *22*). The present tumor contained an 8;22 translocation, one of the variant translocations that has been described in 20 percent of patients with Burkitt's lymphoma (*26*). Lenoir and colleagues reported that in seven of seven cell lines or tumors with an 8;22 translocation in which light chains could be detected, they were invariably of the λ type. Conversely, in three of three lines with a 2;8 translocation and detectable light chains, they were invariably of κ type (*15*). The cell lines described here clearly differ in that although they have an 8;22 translocation, they express immunoglobulin of κ light chain type on the cell surface and secrete κ light chains into the culture medium.

The breakpoints in the translocations associated with Burkitt's lymphoma are in banding regions known to contain the immunoglobulin genes (14q32, 22q11-12, and 2p12), while the c-*myc* gene is located in the distal region of the long arm (q) of chromosome 8 (*2*, *27–30*). There is direct evidence that c-*myc* is translocated with this distal segment into chromosome 14 in a number of cell lines from studies of mouse-human somatic cell hybrids (for example, with Daudi and P3HR1) (*2*, *24*, *25*). In several other cell lines a Bam HI fragment containing the c-*myc* gene comigrates electrophoretically with a fragment containing immunoglobulin heavy chain DNA sequences (*31*, *32*). These findings suggest that the translocated c-*myc* may come under the transcriptional control of immunoglobulin genes. Support for this possibility has been provided by experiments with somatic cell hybrids and chromosome selection. When the Burkitt 14q+ chromosome containing the translocated c-*myc* gene is present in a mouse myeloma background, high levels of human c-*myc* transcripts are expressed. The normal c-*myc* on chromosome 8, however, is silent in similar hybrid cells (*33*). Further, c-*myc* RNA is expressed at low levels or not at all by either chromosome 14q+ or chromosome 8 in a mouse fibroblast background. We have also recently demonstrated a simultaneous increase in both C_μ messenger RNA and c-*myc* RNA when the cell line JD38, containing an 8;14 translocation, is incubated with phorbol ester (*34*), a finding that is consistent with coordinate regulation of c-*myc* and C_μ.

The association between a specific translocation (2;8 or 8;22) and light chain

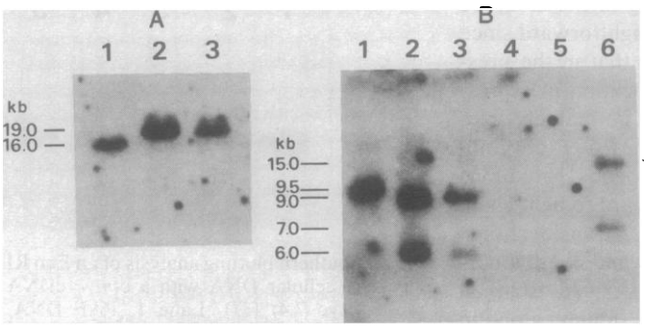

Fig. 4. (A) Southern blotting analysis of Bam HI–digested cellular DNA with a 1.2 kb C_μ genomic clone (*24*). Lane 1, SV40-transformed PAF human fibroblast DNA. Lanes 2 and 3, PA682 PE1 and BM1 DNA. The 16-kb germ line C_μ gene is detected in PAF cells; the two Burkitt lines contain a single rearranged C_μ gene. The C_μ gene on the other chromosome has been deleted. (B) Southern blotting analysis of Bam HI digest of cellular DNA with a C_κ gene probe. Lane 1, PAF DNA. Lanes 2 and 3, PA682 PE1 and BM1 DNA, respectively. Lanes 4 and 5, DNA from LY67 and BL2 Burkitt lymphoma cells carrying the t(8;22) translocation. Lane 6, Jl Burkitt lymphoma cells carrying the t(2;8) translocation (*24*). The germ line arrangement is present in PAF cells. The PA682 lines and Jl cells show rearrangement of both κ genes whereas both genes are deleted in the two other cell lines (LY67 and BL2) with an 8;22 translocation. The probe used was a C_κ complementary DNA in M13 mp7 that is equivalent to codon 115 until the polyadenylated tail of the messenger RNA (*40*).

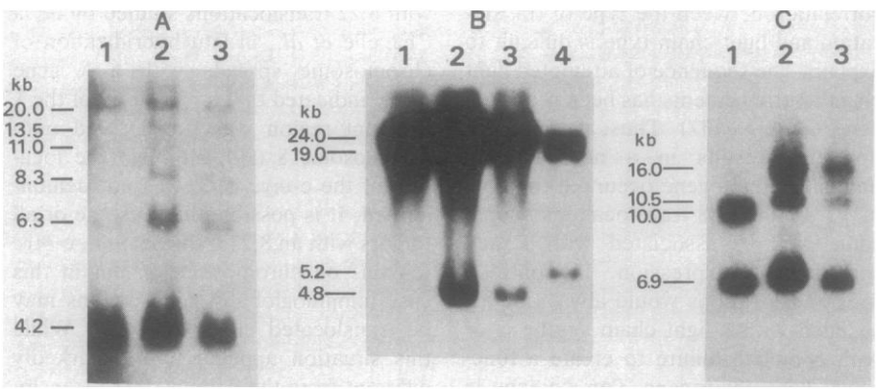

Fig. 5. Southern blotting analysis of Burkitt lymphoma DNA with a C_λ probe (Chr22 λ5). This is a C_λ genomic clone in λwes that contains a 8.0-kb Eco RI fragment which includes Ke^-OZ^- and Ke^-OZ^+. (A) Lane 1, PAF DNA. Lanes 2 and 3, PA682 PE1 and BM1 DNA, respectively, cut with Bam HI. No differences in the cell lines are detected with this enzyme. (B) Lane 1, PAF DNA. Lanes 2 and 3, PA682 PE1 and BM1 DNA, respectively. Lane 4, LY67 DNA. Cut with Eco RI. The same rearranged C_λ band is present in PA682 PE1 and BM1, and LY67 (which contains an 8;22 translocation) also has a rearranged C_λ band. The germ line configuration is shown in PAF DNA. (C) Lane 1, PAF DNA. Lanes 2 and 3, PA682 PE1 and BM1 DNA, respectively, cut with Hind III. A new 16-kb band is observed in PA682 PE1 and BM1 cells compared to the PAF cells (germ line configuration).

Table 1. Characteristics of the PA682 cell lines. For immunological studies, the intensity of fluorescence (based on the mean channel number in a cytofluorographic profile) is indicated by the number of plus signs. Instrument gain was standardized by means of fluorescent microspheres.

Characteristic	PE1	BM1	PB
Secreted immunoglobulin	IgMκ	IgMκ	IgMκ
Surface immunoglobulin:			
μ	+	+	+
κ	+	+	+
λ	−	−	−
CALLA (J5)	+	+	+
B1	++	++	++
B2	+	+	+
HLA	++++	++++	++++
HLA-DR	+	+	+
β2 Microglobulin	+++	+++	+++
Complement receptors	10 to 16 percent	10 to 16 percent	15 to 20 percent
EBNA	+	+	+
Viral capsid antigen	10 to 15 percent	7 to 10 percent	7 to 10 percent
Herpesvirus particles	+	+	+
Transformation of cord blood lymphs	+	+	+
Infection of HR1 cells	+	+	+
Chromosomal translocation	8;22	8;22	8;22

synthesis corresponding to the involved chromosome is not straightforward since there is good evidence that in the presence of an 8;14 translocation μ expression occurs from the normal chromosome 14 rather than the 14q+ chromosome (*24*, *35*). Analogous situations probably occur with regard to light chain expression in 2;8 or 8;22 translocations. In addition, transcripts and even abnormal proteins may be derived from an abortively rearranged immunoglobulin gene (*36*), and the highly expressed c-*myc* oncogene is on the chromosome 14 in which VDJ joining has been unsuccessful (*24*, *35*). Nevertheless, it seems likely that translocation occurs during, or is in some way associated with, rearrangement of immunoglobulin genes—an integral component of the process of B-cell differentiation—since otherwise the correlation between the type of translocation and light chain type is difficult to explain. The sequence of attempted light chain rearrangements has been proposed as κ→κ→λ→λ (*37*). Thus, if translocation that results in a nonfunctional immunoglobulin gene occurs at the time of VJ joining, 2;8 translocations should sometimes be associated with κ and sometimes λ expression. In contrast, 8;22 translocations would always be associated with λ light chain synthesis or with complete failure to create a functional light chain gene. Our findings in the PA682 cell lines indicate either that translocation occurred as a separate event from VJ joining or that the orderly sequence of attempted rearrangement of light chain genes was disturbed. In PA682, one λ gene is rearranged, although κ light chain is synthesized. This is contrary to the expected germ line arrangement of λ genes in κ synthesizing cells unless the λ rearrangement is solely a consequence of the translocation and does not indicate attempted VJ joining. Additional tumors and cell lines with 2;8 or 8;22 translocations should be studied to determine more precisely the frequency of discrepancies between translocations and light chain expression.

The present study also suggests that the c-*myc* oncogene may be activated by its close proximity to an immunoglobulin chain gene in B cells independent of the immunoglobulin genes that are expressed. It will thus be important to identify directly the chromosomal location of the c-*myc* gene and of the λ constant region genes in the PA682 cell lines. However, c-*myc* expression in these lines is similar to that in American Burkitt cell lines with 8;14 translocations and is at least twofold greater than c-*myc* expression in lymphoblastoid lines of nontumor origin (*38*). In two cell lines with 8;22 translocations studied by de la Chapelle *et al.*, in situ hybridization of chromosome spreads with a λ gene probe indicated that a part or all of the λ constant region was translocated onto chromosome 8 (*39*), although the location of the c-*myc* gene was not demonstrated. It is possible that in some or all tumors with an 8;22 translocation, c-*myc* remains on chromosome 8, but in this case immunoglobulin gene regions may be translocated close to c-*myc*. While this situation appears to be markedly different from the demonstrated translocation of c-*myc* into chromosome 14 in cell lines with 8;14 translocations (*24*, *30*), it is of interest that the c-*myc* gene is not always rearranged in the presence of an 8;14 translocation and in such lines the chromosomal location of c-*myc* must be demonstrated directly. In any event, whether c-*myc* is translocated to an immunoglobulin gene region, or vice versa, the net result—altered c-*myc* expression—may be the same. Somatic cell hybrid studies with human chromosomal selection, and in situ hybridization with a c-*myc* gene probe on chromosome spreads should reveal the chromosomal locations of c-*myc* and C_λ in the PA682 cell lines.

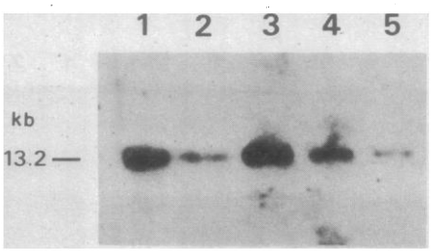

Fig. 6. Southern blotting analysis of an Eco RI digest of cellular DNA with a c-*myc* cDNA probe (Ryc 7.4) (*31*). Lane 1, PAF DNA. Lane 2, P3HR1 cellular DNA. Lanes 3 and 4, PA682 PE1 and BM1 DNA, respectively. Lane 5, DNA from t(2;8) Burkitt lymphoma LY91 cells. In all samples the c-*myc* oncogene is in its germ line configuration. A similar blot after Bam HI digestion showed no c-*myc* rearrangements in all five cell lines (data not shown).

References and Notes

1. P. Gunven, G. Klein, E. Klein, T. Norin, S. Singh, *Int. J. Cancer* **25**, 711 (1982).
2. R. Dalla-Favera *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7824 (1982).
3. L. Zech, U. Hoagland, K. Nilsson, G. Klein, *Int. J. Cancer* **17**, 47 (1976).
4. A. Bernheim, R. Berger, G. Lenoir, *Cancer Genet. Cytogenet.* **3**, 307 (1981).
5. R. Berger *et al.*, *Nouv. Rev. Hematol.* **23**, 39 (1981).
6. E. C. Douglass, I. T. Magrath, E. C. Lee, J. Whang-Peng, *Blood* **55**, 148 (1980).
7. C. L. M. Olweny *et al.*, *J. Natl. Cancer Inst.* **58**, 1191 (1977).
8. I. T. Magrath, in *Cancer in the Young*, A. S. Levine, Ed. (Masson, New York, 1982), pp. 473–574.
9. M. Anderson, G. Klein, J. L. Ziegler, W. Henle, *Nature (London)* **260**, 357 (1976).
10. I. T. Magrath *et al.*, *J. Natl. Cancer Inst.* **64**, 477 (1980).
11. C. W. Berard, M. Green, E. Jaffe, I. T. Magrath, J. L. Ziegler, *Ann. Int. Med.* **94**, 228 (1981).
12. D. Benjamin *et al.*, *J. Immunol.* **129**, 1336 (1982).
13. J. L. Ziegler *et al.*, *Lancet* **1982-II**, 631 (1982).
14. R. S. K. Chaganti *et al.*, *Blood* **61**, 1269 (1983).
15. G. M. Lenoir, J. L. Preud'homme, A. Bernheim, R. Berger, *Nature (London)* **298**, 474 (1982).
16. L. M. Nadler *et al.*, *J. Clin. Invest.* **67**, 134 (1981).
17. L. M. Nadler *et al.*, *J. Immunol.* **126**, 1941 (1981).
18. J. Ritz, J. M. Pesando, J. Notis-McConarty, H. Lazarus, S. F. Schlossman, *Nature (London)* **283**, 58 (1980).
19. Two different mouse monoclonal antibodies to human μ chain (Becton and Dickinson and Cappel) and a goat heterologous antibody to human μ chain (Cappel) were used for detection of surface μ heavy chain expression. The presence of surface immunoglobulin light chain was assessed with two separate mouse monoclonal antibodies each for κ or λ light chains (Becton and Dickinson and Cappel). The remaining antigens were assayed by commercially available monoclonal antibodies. The J5 antibody, recognizing CALLA, was kindly provided by J. Ritz, Boston; B1 and B2 antibodies were obtained from Coulter Corporation; and β2-microglobulin and anti-HLA-DR were from Becton and Dickinson. The mouse monoclonal antibody to human-HLA antibody was purchased from Bethesda Research Laboratories. The presence of antibodies on the cell surface was detected by an indirect immunofluorescence technique with an FITC-coupled goat antibody to mouse IgG (Cappel) that reacted with the mouse monoclonal antibodies and a rabbit antibody to goat IgG (Cappel) that reacted with the goat antibody to human μ chain.
20. Affinity-purified antibodies against μ, γ, or α heavy chains and κ or λ light chains (Cappel) were used to coat plastic microwell plates (Dynatech), and the presence of immunoglobulins was detected by similar antibodies coupled to alkaline phosphatase (Sigma and E.Y.) used at predetermined optimal dilutions. The specificity of all antibodies was confirmed by testing against purified IgM, IgG, IgA, or Bench Jones protein of κ or λ type (Cappel), and these same purified preparations were used to construct standard curves for quantification of secreted immunoglobulin.
21. P. Gerber *et al.*, *J. Natl. Cancer Inst.* **69**, 585 (1982).
22. J. Whang-Peng, E. C. Lee, H. Sieverts, I. T. Magrath, *Blood*, in press.
23. R. A. Taub *et al.*, *Nature (London)* **304**, 172 (1983).
24. J. Erikson, A. Ar-Rushdi, H. L. Drwinga, P. C. Nowell, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 820 (1983).
25. K. B. Marcu *et al.*, *ibid.*, p. 519.
26. G. M. Lenoir, T. Philip, R. Sohier, in *Pathogenesis of Leukemias and Lymphomas: Environmental Influences*, I. T. Magrath *et al.*, Eds. (Raven, New York, in press).
27. J. Erikson, J. Martinis, C. M. Croce, *Nature (London)* **294**, 173 (1981).
28. O. W. McBride *et al.*, *J. Exp. Med.* **155**, 1480 (1982).
29. S. Maleolum *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4957 (1982).
30. I. R. Kirsch, C. C. Morton, K. Nakahara, P. Leder, *Science* **216**, 301 (1982).
31. R. Dalla-Favera, S. Martinotti, R. C. Gallo, J. Erikson, C. M. Croce, *ibid.* **219**, 963 (1983).
32. R. Taub *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7837 (1982).
33. K. Nishikura *et al.*, *ibid.* **80**, 4822 (1983).
34. D. Benjamin *et al.*, in preparation.
35. J. Erikson, J. Finan, P. C. Nowell, C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5611 (1982).
36. T. Seidman and P. Leder, *Nature (London)* **286**, 779 (1980).
37. S. J. Korsmeyer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7096 (1981).
38. R. T. Maguire, T. H. Robins, S. S. Thorgeirsson, C. A. Heilman, *ibid.* **80**, 1947 (1983).
39. A. de la Chapelle *et al.*, *Nucleic Acids Res.* **11**, 1133 (1983).
40. T. Rabbits, in preparation

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