Development of a Second Clonally Discrete Burkitt’s Lymphoma in a Human Immunodeficiency Virus-Positive Homosexual Patient


We have studied, at a molecular level, two small noncleaved cell malignant lymphomas (Burkitt’s type) that were separated by a disease-free interval of 3 years in a patient infected with the human immunodeficiency virus (HIV). The late occurrence of the apparent relapse suggested that the second lymphoma might be caused by a separate malignant transformation in a discrete clone of B cells. Although both tumors expressed the same immunologic surface markers (μk) and carried the same t(8;14) translocation, Southern blot analysis of DNA from each tumor, using specific restriction endonucleases and probes to the c-myc and the immunoglobulin heavy chain loci, demonstrated that the chromosomal breakpoints relevant to the translocations differed between the tumors. This was corroborated by analysis of the immunoglobulin light-chain rearrangements in the two tumors. These observations indicate that the second tumor was not a recurrence of the first but represented the malignant transformation of a different clone of B cells. Thus late relapses of certain malignancies in individuals at high risk may be caused by the malignant transformation of discrete cell clones (i.e., induction of a new tumor).

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INDIVIDUALS with human immunodeficiency virus (HIV) infection are at increased risk to develop malignant lymphomas, particularly small noncleaved lymphomas. Moreover, a large proportion of HIV-positive patients with lymphomas, even if they respond favorably to treatment, develop an early relapse or succumb to fatal infections as a consequence of the added immunosuppression induced by chemotherapy. While a proportion of such patients may be cured of their neoplasms, their heightened susceptibility to the development of lymphoma probably persists so that there is a theoretic possibility that a new lymphoma will develop (i.e., a second malignant transformation will occur in a previously nonmalignant cell). This possibility exists for all individuals with cancer but becomes clinically relevant only in those who are at extremely high risk for a specific cancer, a category that includes patients with HIV infection. Moreover, the occurrence of a new tumor, as opposed to recurrence of the former tumor, is only verifiable in neoplasms in which there is a specific clonal marker. Such a marker is provided in tumors in which structural genetic alterations occur during tumorigenesis, or, as is the case for neoplasia of the immune system, when detectable genetic rearrangements are necessary for the differentiation and function of a cell. Small noncleaved lymphomas (SNCL) fall into this category. Not only are there rearrangements of immunoglobulin genes, since SNCL is a B-cell tumor, but structural alterations occur in the c-myc oncogene as a consequence of the non-random translocations—t(8;14), t(8;22) and t(2;8)—associated with this tumor.

The authors report here a case of an HIV-positive homosexual patient in whom the sequential development of two clonally discrete Burkitt’s lymphomas, the consequence of separate chromosomal translocations, confirms this theoretic possibility. In this case simple surface marker analysis or karyotyping would have been inadequate to determine whether the second tumor was a recurrence of the first or a quite separate malignant clone. Therefore a detailed molecular analysis was required to demonstrate that discrete, sequential translocations and, therefore, presumably malignant transformations had occurred.

CASE REPORT

The patient is a 27-year-old bisexual male, and his clinical history has, in part, been previously reported. At age 23 he presented to the National Cancer Institute with a 2-week history of increasing abdominal girth, a 20-pound weight gain, night sweats, and progressive shortness of breath. His prior history was remarkable for multiple sexual partners, 8 months of amyl nitrate use, gonococcal urethritis, hepatitis A, and, in 1977, meningitis. Physical examination and staging evaluations revealed a 4 x 6-cm pelvis mass, ascites, and bilateral pleural effusions. A diagnosis of Burkitt’s lymphoma was established by biopsy of the rectal mass. Pleural and ascitic fluids were positive for tumor, but spinal fluid and bone marrow aspirates and biopsies were normal. The patient’s serum was found to be positive for HIV by enzyme-linked-immunosorbent assay (ELISA) and Western Blot analyses. The tumor carried an 8:14 translocation (Fig 1A) and expressed surface immunoglobulins (ELISA) and Western Blot analyses. The tumor carried an 8:14 translocation (Fig 1A) and expressed surface immunoglobulins with μκ chains. Induction therapy with cyclophosphamide was complicated by acute tumor lysis and renal failure as well as pulmonary infiltrates with respiratory failure requiring mechanical ventilation. The patient subsequently completed 12 cycles of chemotherapy (NCI protocol 77-04) with cyclophosphamide, Adriamycin, vincristine, prednisone, and high-dose methotrexate as well as intrathecal methotrexate and cytarabine. A prompt and complete remission was achieved, and therapy was terminated approximately 1 year from diagnosis.

The patient remained well and in complete remission without further therapy for 27 months, during which time he was followed closely at the National Cancer Institute. Thir-
of detectable disease. He has not had other clinical manifestations of HIV infection.

MATERIALS AND METHODS

Cytogenetics. Chromosome preparations of the 1-day and 2-day cultures of the tumor cells were made according to the method of Moorehead et al.4 One of the resulting slides was stained with the standard Giemsa stain, and the remainder were G-banded using a modification of the trypsin method of Seabright.7 The metaphases were scored for modal number and aberrations and karyotyped using established nomenclature.8

Molecular Genetic Characterization. DNA was obtained from (1) tumoral ascites obtained on initial presentation, and (2) the retromandibular tumor at recurrence. A cell line obtained from the original tumor was also analyzed but did not differ in any respect from the original fresh tumor cells, and these data are not, therefore, presented here. DNA was extracted by cell lysis, digestion with pronase, extraction with phenol/chloroform, and precipitation with ethanol.9 DNA from these sources was digested with five different restriction endonucleases: EcoRI, HindIII, PstI, PvuII, and BamHI using protocols provided by the supplier (Bethesda Research Laboratories, Bethesda, MD), and fractionated by 0.8% agarose gel electrophoresis. The DNA was transferred to nylon filters (S&S) by standard Southern blotting procedures. The filters were then hybridized with the following probes (Fig 2): Cu—EcoRI-EcoRI9, third exon c-myc—Clal-EcoRI17; first exon c-myc—PvuII-PvuII.12 JH—BamHI-HindIII10; J1—EcoRI-EcoRI11, C1—EcoRI-EcoRI11, and C2—BamHI-BamHI.11 The probes were labeled with 32P by a standard nick-translation reaction.9 A 50% formamide, 10% dextran solution was used for hybridization at 42°C overnight. The filters were each washed once at room temperature, 37°C and 65°C, in 6 x SSC-0.1% sodium dodecyl sulfate (SDS), 1 x SSC-0.5% SDS, and 0.1 x SSC-1% SDS, respectively, and exposed to x-ray film for 2 to 5 days.

RESULTS

Cytogenetics. Figure 1 shows the karyotypes of both tumors. Both were 46,XY,t(8;14)(q24;q32). All the mitoses examined from the original tumor also carried an unbal-

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Fig 1. (A) Karyotype of the initial presentation: 45,XY,t(8;14)(q24;q32),der(4)[t(1;4)(q21-q44;pter),der(21)[t(21;22)(p11;q11)]. The loss of one chromosome 13 in this metaphase was a random event and was not seen in other metaphases studied. (B) Karyotype of the recurrent tumor: 46,XY,t(8;14)(q24;q32). ty-nine months after his initial presentation he complained of left submandibular swelling, which had been present for 1 week. Physical examination revealed a 2.5 × 4.0-cm mass that decreased in size in the course of a week. However, 2 months later the mass enlarged and at this time measured 3.5 × 7 cm. An excisional biopsy of the mass revealed Burkitt’s lymphoma (41 months since the initial diagnosis and 29 months after chemotherapy). Physical examination, complete radiographic evaluation, as well as bone marrow and spinal fluid analysis failed to show other sites of disease. The tumor cells exhibited an 8:14 translocation (Fig 1B) and the surface phenotype was again mu-k. The patient was treated with six cycles of the same chemotherapy that he received originally. He completed this treatment without major complications and is currently off therapy at 11 months, and free

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Fig 2. Map of the human c-myc (chromosome 8) and immunoglobulin heavy-chain (chromosome 14) loci that are usually involved in the 8:14 translocations in Burkitt’s lymphoma. Restriction sites as well as the segments used for probes are shown. 1myc, first exon c-myc; 3myc, third exon c-myc.
ancest translocation t(1;4) (q21-q44;qter) that resulted in a duplication of 1q21-44 as well as a translocation t(21;22) (p11;q11). These abnormalities were not present in the second tumor.

**Molecular genetics characterization.** Autoradiographs of Southern blots obtained with the various restriction enzyme/DNA probe combinations are shown in Fig 3. Both tumors showed c-myc rearrangements when DNA was digested with each of four different enzymes and hybridized to probes derived from the first and third c-myc exons. In each tumor the first exon and third exon sequences were in different restriction fragments (using HindIII and EcoRI), indicating a breakpoint within the c-myc gene. However, the size of the rearranged c-myc band differed between the two tumors with each of the restriction enzyme/c-myc probe combination. The immunoglobulin heavy- and light-chain loci were also rearranged in both tumors, and once again the rearrangements detected in each locus were different in the two tumors (Fig 3). In the first tumor the Ck locus appeared to be in close contiguity with the third exon of c-myc, as evidenced by comigration of two sequences in the same 19 kb HindIII rearranged fragment. This same fragment was intact in the second tumor. The light chain rearrangement was detected with the Jα probe in blots with Pst I-digested DNA and again, a different rearrangement of the Kappa locus was seen. DNA digested with BamHI and EcoRI and hybridized with the Ck or Cα probes failed to show any rearrangements in the two tumors.

**Virologic studies.** All three samples were Epstein-Barr virus (EBV)-positive by hybridization of BamHI-digested DNA with a radiolabeled EBV probe (BamHI k fragment, data not shown). Culture of tumor cells for HIV and human B-lymphotropic virus (HBLV) was negative.

**DISCUSSION**

This HIV-infected homosexual patient presented with a late recurrence of a Burkitt’s lymphoma 3 years after the original presentation and 29 months after completing successful chemotherapy. The presence of a predisposing factor (HIV infection) for this kind of malignancy and the timing of the recurrence (unusually late for relapsed Burkitt’s lymphoma) suggested the possibility that this relapse represented a new malignant transformation in a previously normal B-cell clone in this patient. We provide conclusive evidence for this hypothesis. Both tumors were SNCL, and each carried an 8:14 translocation. Other karyotypic markers present in the first tumor were absent in the second. The molecular analysis of the c-myc and IgH loci demonstrated that the chromosomal breakpoints were different in each tumor, indicating that separate translocations occurred. In so far that altered regulation of the c-myc gene, brought about by the 8:14 translocation, is believed to be an essential (though not necessarily sufficient) component of pathogenesis, this indicates that each tumor was induced by a separate malignant transformational event. A formal demonstration that the second tumor could not have been derived from the first was provided by this analysis. In the first tumor the breakpoint in chromosome 14 was within the HindIII fragment encompassing the μ switch region (Fig 2), leading to the detection of a rearrangement of this fragment and comigration of Ck and the third exon of c-myc in HindIII blots. In the second tumor this HindIII fragment was not rearranged, indicating that the breakpoint was outside this HindIII fragment. It is difficult to conceive of a mechanism, other than a second translocation in a discrete B-cell clone, whereby a rearranged fragment could return to germ-line status. The different rearrangements found in the light-chain locus further reafirm that each tumor is clonally distinct in spite of the fact that both made kappa light chains, so that determination of the type of light chain was insufficient to document this.

These observations are of importance, since they represent, as far as we are aware, the first definitive description of the occurrence of two separate translocations of the same type, giving rise to two clonally discrete tumors in the same patient. Previous observations suggesting that an individual patient can develop two separate African Burkitt’s lymphomas have been made on the basis of glucose-6-phosphate dehydrogenase (G6PD) isoenzyme phenotypes. In this report a patient developed progressive disease that was reported as having a different isoenzyme phenotype from the original and recurrent tumors. Our observations suggest that any patient at very high risk to develop a specific neoplasm could develop a new tumor from a separate transformational event. This possibility doubtless explains very late relapse in at least some such patients and, apart from its theoretic interest, has practical significance. In the patient described here, the demonstration that the second tumor was geneti-
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...cally distinct supported the decision to treat the patient with similar therapy to that used for the original tumor—there is no reason to believe that the second tumor would have developed acquired resistance to chemotherapy, since it was not exposed to the chemotherapeutic agents used to treat the first tumor. The patient received treatment of shorter overall duration for his second tumor because of its limited extent. The long-term prognosis must remain guarded due to his predisposition toward the development of SNCL.

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