Molecular Analysis of Burkitt’s Leukemia in Two Hemophilic Brothers With AIDS


In two hemophilic brothers infected by the human immunodeficiency virus (HIV), Burkitt’s leukemia developed within 1 year. Both patients were treated by aggressive chemotherapy, and both are still in complete remission for 23 and 14 months, respectively. Sera from both brothers contained anti-HIV antibodies. However, DNA extracted from the tumor cells, when analyzed by Southern blot using a cloned HIV probe, did not reveal HIV-related sequences. Hybridization experiments with an Epstein-Barr virus (EBV) probe revealed the presence of EBV-specific sequences in the tumors’ DNA. In both patients’ tumors rearranged c-myc genes were found. The rearrangements occurred in both genes 3’ to the third exon of c-myc, thereby suggesting that a variant chromosomal translocation took place in both cases. Indeed, karyotype analysis of the malignant cells of one of the patients revealed the variant t(2;8) translocation. In contrast to the majority of Burkitt’s tumors carrying this translocation, which are light-chain producers, cells of our patient expressed λ chains. Furthermore, in both cases the lymphoblasts carried IgG on the surface, again an unusual finding in Burkitt’s tumors. Finally, because both patients had an identical HLA phenotype, the role of genetic factors in the development of such tumors should be considered.

ALIGNANCIES LIKE KAPOSI’S sarcoma or lymphoma are known manifestations of the acquired immunodeficiency syndrome (AIDS). The B cell lymphomas in these patients are frequently high-grade B cell malignancies, involve extra nodal sites, and run an aggressive course. Most cases of B cell tumors have been reported in homosexuals and drug addicts, whereas only a few have malignancies, involve extra nodal sites, and run an aggressive course in these patients are frequently high-grade B cell immunodeficiency syndrome (AIDS). The B cell lymphoma A who had been treated for years with factor VIII concentrates. In January 1984, he started to suffer from recurrent infections, and in December 1985, he presented with severe skeletal pain, an upper respiratory infection. In April 1985, anti-HIV antibodies were first assayed at that time and found to be present. A cervical lymph node biopsy specimen taken on March 1985 was diagnosed as diffuse small noncleaved (Burkitt’s) lymphoma. A bone marrow aspirate revealed infiltration by lymphoblasts with an L3 morphology that amounted to 30% of the cells. Treatment was begun according to a modified National Institute of Health protocol 7704. After 2 months of receiving chemotherapy, a complete remission was achieved, and treatment was stopped in December 1985. He has remained since then in good general condition except for recurrent infections.

Patient no. 2 is a 16-year-old boy with severe hemophilia A who had been treated for years with factor VIII concentrates. In March 1983 he presented with a rapidly disseminating cutaneous and visceral varicella-zoster virus (VZV) infection. He had a 1-year history of weight loss, cervical lymphadenopathy, splenomegaly, and oral candidiasis. In January 1985 the lymphadenopathy progressed and became generalized. Anti-human immunodeficiency virus (HIV) antibodies were first assayed at that time and found to be present. A cervical lymph node biopsy specimen taken on March 1985 was diagnosed as diffuse small noncleaved (Burkitt’s) lymphoma. A bone marrow aspirate revealed infiltration by lymphoblasts with an L3 morphology that amounted to 30% of the cells. Treatment was begun according to a modified National Institute of Health protocol 7704. After 2 months of receiving chemotherapy, a complete remission was achieved, and treatment was stopped in December 1985. He has remained since then in good general condition except for recurrent infections.

Hence, we report two hemophilic brothers with AIDS who developed Burkitt’s leukemia within 1 year. The role of viral infections and genetic factors in the evolution of lymphomas in such patients will be discussed.

PATIENTS AND METHODS

Patients. Patient no. 1 is a 16-year-old boy with severe hemophilia A who had been treated for years with factor VIII concentrates. In March 1983 he presented with a rapidly disseminating cutaneous and visceral varicella-zoster virus (VZV) infection. He had a 1-year history of weight loss, cervical lymphadenopathy, splenomegaly, and oral candidiasis. In January 1985 the lymphadenopathy progressed and became generalized. Anti-human immunodeficiency virus (HIV) antibodies were first assayed at that time and found to be present. A cervical lymph node biopsy specimen taken on March 1985 was diagnosed as diffuse small noncleaved (Burkitt’s) lymphoma. A bone marrow aspirate revealed infiltration by lymphoblasts with an L3 morphology that amounted to 30% of the cells. Treatment was begun according to a modified National Institute of Health protocol 7704. After 2 months of receiving chemotherapy, a complete remission was achieved, and treatment was stopped in December 1985. He has remained since then in good general condition except for recurrent infections.

Patient no. 2 is a 21-year-old hemophiliac and the elder brother of patient 1 who had also been treated for many years with factor VIII concentrates. In January 1984, he started to suffer from recurrent upper respiratory infections. In April 1985, anti-HIV antibodies were first found. Concomitantly, a persistent lymphopenia (0.5 × 10^9/L) and thrombocytopenia (35 to 85 × 10^9/L) were observed. In December 1985, he presented with severe skeletal pain, and bone marrow biopsy material and aspirates showed heavy infiltration by L3 lymphoblasts that amounted to 80% of the cells. Computed tomography of the abdomen did not reveal any masses. The patient was treated by a modified German Multicenter Trial (BFM) protocol for B cell leukemia, and 1 month later he was in complete remission. Treatment was stopped after 4 months, and since April 1986 he remains in complete remission. An informed consent for bone marrow aspiration and chemotherapy administration was obtained from both patients and their parents.

Methods. Antibodies to HIV were assayed by an enzyme-linked immunosorbent method and confirmed by Western blot with a commercial kit (Du-Pont, Wilmington, DE) according to the manufacturer’s instructions. Antibodies to Epstein-Barr virus (EBV) early antigen (EA) and viral capsid antigen (VCA) were detected by an indirect immunofluorescence test. Immunophenotyping of the tumor cells, HLA typing, and karyotype analysis were performed as described. DNA was extracted from the tumor cells, digested with the appropriate restriction enzymes, separated according to size by electrophoresis in 0.7% agarose, and analyzed by Southern blot hybridization with the relevant radiolabeled probe. The c-myc probe was the 1.4-kilobase (kb) Clal-EcoRI insert of the plasmid pMC4l-3RC containing exon 3 of c-myc. The EBV probe contains the 4.8-kb BamHI K fragment of EBV, which was cloned in pBR322 and encodes for the first EBV-associated nuclear antigen. The cytomegalovirus (CMV) probe pCM5018 contains the EcoRI J fragment that encodes for the 1.9-kb major immediate early RNA. The VZV probe contains the EcoRI A fragment. The herpes simplex virus (HSV1) probe (pSG28) contains the EcoRI E to K fragments cloned in pBR325. The probe for HIV was the pPol (Du-Pont), derived from the human T-lymphotropic virus III clone λBH10 and contains the 4.9-kb BglII-BglII fragment.

From the Institute of Hematology and Central Virology Laboratory, Chaim Sheba Medical Center, Tel-Hashomer, and Sackler School of Medicine, Tel-Aviv University, Israel.


Supported in part by the Moise and Frida Eskenasy Institute for Cancer Research (G.R.). Dr Rechavi is a Fellow of the David Laser Institute for Cancer Research (G.R.).

Address reprint requests to G. Rechavi, MD, PhD, Institute of Hematology, Chaim Sheba Medical Center, Tel-Hashomer, Israel 52621.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

0006-4971/87/7006-0162$03.00/0

Blood, Vol 70, No 6 (December), 1987: pp 1713-1717

1713
RESULTS

Antibody assays. Serum samples from both brothers were positive for anti-HIV antibodies. Figure 1 depicts the Western blots confirming the serological tests. Both sera showed strong reaction with several HIV proteins, but differences in the pattern of reactivity could be demonstrated: the serum of patient no. 1 did not react against the gag-gene product p17 or against p51,26,27 The results of determination of the titers of antibodies to EBV-EA and -VCA are summarized in Table 1. The antibody response is consistent with reactivation of EBV infection, as can be found in many AIDS patients.

Both patients had antibodies in high titers to CMV, VZV, and HSV.

Immunophenotype analysis. Bone marrow lymphoblasts from patient no. 1 were HLA-DR+, CALLA-, My906-, WT1-, OKT3-, OKT4-, OKT6-, OKT8-, OKT11-, Tac-, γ+, μ-, δ–, κ–, and λ–.

DNA hybridization studies. DNA extracted from the tumor cells was assayed by Southern blot analysis using as a probe a cloned HIV DNA fragment that contains the pol gene. No HIV-related sequences could be demonstrated (data not shown). Similarly, Southern blot hybridization to CMV, VZV, and HSV probes did not detect hybridizing sequences to any of them (data not shown). DNA hybridization with a 32P-labeled EBV probe revealed the presence of EBV-specific sequences in DNA extracted from the tumors of both siblings (Fig 2).

Figure 3 depicts the Southern blot hybridization to a 32P-labeled c-myc probe. In both DNA samples extracted from the tumor tissues, rearranged c-myc bands were found. In the tumor of both siblings, the rearranged c-myc bands were found in the HindIII digest but not in the EcoRI digest. The change in the length of the HindIII-HindIII c-myc fragment without alteration of the EcoRI-EcoRI c-myc fragment localizes the breakpoint to the EcoRI-HindIII fragment downstream to exon 3 of c-myc (Fig 3, insert).

<table>
<thead>
<tr>
<th>Table 1. Titers of Antibodies to EBV-EA and -VCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient no. 1</td>
</tr>
<tr>
<td>12/22/82</td>
</tr>
<tr>
<td>5/30/83</td>
</tr>
<tr>
<td>3/15/84</td>
</tr>
<tr>
<td>9/12/84</td>
</tr>
<tr>
<td>3/14/85*</td>
</tr>
<tr>
<td>3/10/86</td>
</tr>
<tr>
<td>EVB-EA</td>
</tr>
<tr>
<td>&lt;1:5</td>
</tr>
<tr>
<td>1:5</td>
</tr>
<tr>
<td>&lt;1:5</td>
</tr>
<tr>
<td>&lt;1:5</td>
</tr>
<tr>
<td>1:10</td>
</tr>
<tr>
<td>1:10</td>
</tr>
<tr>
<td>1:280</td>
</tr>
<tr>
<td>1:80</td>
</tr>
<tr>
<td>EVB-VCA</td>
</tr>
<tr>
<td>1:10</td>
</tr>
<tr>
<td>1:10</td>
</tr>
<tr>
<td>1:280</td>
</tr>
<tr>
<td>1:640</td>
</tr>
<tr>
<td>1:80</td>
</tr>
</tbody>
</table>

\*At diagnosis of Burkitt’s leukemia.

Bone marrow lymphoblasts from patient no. 2 were OKT11–, γ+, μ–, δ–, κ–, and λ–.

**Fig 1.** Western blot analysis of sera obtained from both patients. Control samples are shown on the right. On the left: P1, patient no. 1; P2, patient no. 2. Corresponding viral proteins (p) and glycoproteins (gp) are shown in the middle.
Hind I

5.
EcoRI

'-a

1kb

R!

k'

'Hind III

3.

Fig 3. C-myc rearrangements in DNA samples from the patients' leukemic cells. DNA samples (15 μg) were digested by EcoRI (left) and HindIII (right). DNA fragments were separated by electrophoresis and transferred to nitrocellulose paper. The paper was hybridized to a 32P-labeled c-myc probe. Hybridizing bands corresponding to c-myc germine genes are marked by dashes. Horizontal arrows indicate the rearranged c-myc genes detected in HindIII patient no. 1 (rc-myc 1) and patient no. 2 (rc-myc 2). The insert at the bottom depicts the restriction enzyme map of the human c-myc gene. The black rectangles 1, 2, and 3 correspond to exon 1, 2, and 3 of c-myc. Short vertical arrows point to the restriction sites of EcoRI and the long vertical arrows to the HindIII restriction sites. The interrupted line denotes the HindIII-EcoRI fragment that contains the breakpoints in both rc-myc genes. The open rectangle illustrates the c-myc probe (Clal-EcoRI fragment containing exon 3).

Karyotype and HLA studies. Karyotype analysis was performed on the malignant cells of patient no. 2. A variant translocation t(2;8) was found (Fig 4), thereby suggesting the involvement of the chromosomal loci carrying the immunoglobulin κ light-chain genes and the cellular oncogene c-myc.28,29

The HLA phenotypes of the two affected brothers were identical: A11, A29, B53, B7, Cw2, Cw4, DR6, DR10.

DISCUSSION

The two hemophilic brothers developed AIDS-associated Burkitt's leukemia during the same year, and their tumor DNA contained EBV genomes and had rearranged c-myc. Both patients are still in complete remission 23 and 14 months, respectively. This favorable response in Burkitt's leukemia, especially in immunocompromised hosts, is surprising in view of the usually dismal course of the disease.30

In AIDS two factors may predispose the patients to B cell proliferation: HIV and EBV infections. It has been reported that HIV can cause direct B cell proliferation.31 Furthermore, in some animal tumors, a direct role for the retroviral genome in the activation of cellular oncogenes has been demonstrated.32,33 Therefore, we searched for HIV sequences in the patient's tumors and were able to confirm the absence of HIV genome in the tumors of both patients, as previously reported by Groopman et al and Pelicci et al.34,35 It seems, therefore, that the HIV genome does not contribute directly to the malignant transformation.

On the other hand, the finding of the EBV sequences in the tumors suggests a role for this virus in the pathogenesis of AIDS-associated B cell lymphomas similar to other immunocompromised hosts.36-40

Fig 4. A representative karyotype of a bone marrow tumor cell from patient no. 2. The translocated chromosome 2 and 8 are indicated by arrows.
Most Burkitt's lymphomas analyzed thus far were found to carry a rearranged c-myc gene,41,42 and the two tumors described here are no exception. In lymphomas carrying the classic t(8:14) translocation, the chromosomal breakpoints on chromosome 8 are 5' to the two coding exons (2 and 3) of the c-myc oncogenes. In lymphomas carrying the variant translocations t(8:22) and t(2:8), the breakpoints are 3' to exon 3 of c-myc. Our studies locate the breakpoints at 3' of exon 3 of c-myc, thereby suggesting that in both our patients variant translocations are responsible for the c-myc activation. Karyotype analysis of the malignant cells of patient 2 indeed revealed the existence of t(2;8). Such variant translocations are found only in a minority of Burkitt's lymphomas, and their occurrence in the reported two brothers is interesting. Cytogenetic studies have been performed only in a small number of AIDS-associated lymphomas, and three cases of the variant t(8:22) translocation were reported in such tumors.43-46 Analysis of more lymphomas from AIDS patients are necessary to conclude whether the occurrence of variant translocations is more prevalent in this group. The pattern of antibodies to EBV in our patients as well as other immunocompromised hosts is consistent with reactivation of a latent virus. In African Burkitt's patients, tumors develop at a young age where the expanded pool of activated B cells at risk for c-myc rearrangement is composed mainly of IgM-producing lymphocytes. Therefore, the majority of Burkitt's lymphomas express IgM.47 In the patients with AIDS, as expected in secondary response, there is a large pool of IgG-transformed lymphocytes, and therefore it is not unexpected that some of the tumors will be IgG producers as in our cases.

It has been shown by Lenoir et al48 that Burkitt's tumors and cell lines carrying the t(2;8) translocation usually express k light chains whereas those with the t(8;22) translocation synthesize 8 light chains. It was suggested, therefore, that the type of c-myc transposition is in correlation with the level of differentiation of the B cell.49 Lymphoblasts of patient no. 2 are unusual in that they carry t(2;8) but express 8 light chains. Similarly, Magrath et al reported that the lymphoma cells of a homosexual with AIDS and t(8;22) expressed k light chains.43 Taken together, the expression of IgG, the possibly higher incidence of variant translocations, and the lack of correlation between the type of light-chain expression and type of translocation suggest that the sequence of events resulting in B cell tumors in these patients may be different from that of African Burkitt's lymphomas. The two brothers reported on here had an identical HLA phenotype, and although this could have been a mere coincidence, it is tempting to speculate that genetic factors played an important role in the infection with HIV49-51 and in the evolution of the lymphatic malignancy.52-54

ACKNOWLEDGMENT

DNA probes were kindly donated by Drs R.C. Gallo, Miller, B. Fleckenstein, S. Strauss, and M. Levine. We are indebted to Rivka Amit for expert manuscript preparation and to Nili Shaked and Michael Arieli for preparation of the graphic presentation. We are grateful to Dr Shlomit Orgad, Fanny Holtzman, Miriam Biniaminov, Esther Rosenthal, and Esther Rosner for their help.

REFERENCES

AIDS-ASSOCIATED BURKITT'S LEUKEMIA


49. Pollack MS, Saffi B, Dupont B: HLA-DR5 and DR2 are susceptibility factors for acquired immunodeficiency syndrome with Kaposi's sarcoma in different ethnic subpopulations. Dis Markers 1:135, 1983


Molecular analysis of Burkitt's leukemia in two hemophilic brothers with AIDS

G Rechavi, I Ben-Bassat, M Berkowicz, U Martinowitz, F Brok-Simoni, Y Neumann, A Vansover, T Gotlieb-Stematsky and B Ramot