

## Pathogenesis of B Cell Lymphoma in a Patient With AIDS

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Lymphoma occurs at increased frequency in patients with the acquired immunodeficiency syndrome (AIDS). We studied, using serologic and molecular techniques, one such lymphoma for (a) evidence of infection with human T lymphotropic virus, type III (HTLV-III), and Epstein-Barr virus (EBV), (b) monoclonal rearrangement of immunoglobulin and T cell receptor genes, and (c) rearrangement of the *c-myc* oncogene. Immunoglobulin and T cell receptor gene studies demonstrated that the tumor was of monoclonal B cell origin. Similar to cases of Burkitt's lymphoma

unrelated to AIDS, there were DNA sequences in the lymphoma that hybridized to EBV-specific probes and demonstrated evidence of *c-myc* rearrangement. HTLV-III sequences were not detected in the malignant B cells. The pathogenesis of some B cell neoplasms in patients with the syndrome may involve transformation by EBV and deregulation of oncogene expression without direct infection of the malignant B cells by HTLV-III.

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**T**HE ACQUIRED IMMUNODEFICIENCY syndrome (AIDS) is a transmissible disorder etiologically linked with infection by human T cell lymphotropic virus, type III (HTLV-III).<sup>1-3</sup> The major clinical manifestations of AIDS include opportunistic infections or opportunistic neoplasms, such as Kaposi's sarcoma and central nervous system lymphoma.<sup>4</sup> Recently, B cell lymphomas outside the central nervous system have been described in individuals with AIDS or among homosexual men epidemiologically at risk for the disorder.<sup>5-9</sup> We have studied the roles of HTLV-III, the Epstein-Barr virus (EBV), and the *c-myc* oncogene in the pathogenesis of a B cell lymphoma in a man with AIDS. Immunoglobulin plus T cell receptor gene studies were used to confirm monoclonal B cell origin. Our findings suggest that EBV potentiates the development of B cell neoplasia, while HTLV-III sets the immunosuppressive milieu but does not appear to infect the malignant B cells directly. Rearrangement of the *c-myc* oncogene occurred in this case similar to rearrangement in Burkitt's lymphoma unrelated to AIDS.

### MATERIALS AND METHODS

The patient was a 43-year-old Hispanic man whose risk factor for AIDS was 20 years of multiple homosexual contacts. He was diagnosed as having AIDS by biopsy of a cutaneous lesion that revealed Kaposi's sarcoma. His major clinical manifestations of

AIDS included unexplained fevers, diarrhea, and progressive dementia. No enteric pathogens or parasites were identified in his stool and an upper and lower endoscopy revealed Kaposi's sarcoma. The etiology of his dementia was not determined. Lumbar puncture revealed normal glucose of 58 mg/dL, elevated protein of 78 mg/dL, and 10 mononuclear cells per microliter. CT scan of the brain showed generalized atrophy, cultures of cerebrospinal fluid for bacteria, fungi, and mycobacteria were negative, and cytology was unremarkable. The patient died and an autopsy was performed. Sera were collected at multiple times during the course of his illness, and tissues were immediately cryopreserved at the time of death.

Complete blood count, total lymphocyte count, and T cell subsets were performed as previously described.<sup>10</sup> Antibodies to EBV early antigen, nuclear antigen, and viral capsid antigen were measured as previously described.<sup>11</sup> Antibodies to HTLV-III were measured by an enzyme-linked immunosorbent assay and Western electroblot technique.<sup>12</sup> Multiple sections of the B cell lymphoma were studied for EBV nuclear antigen using fluorescent techniques.<sup>3</sup> For immunoglobulin and T cell receptor gene studies, DNA was prepared from a biopsy specimen of the lymphoma, digested with the appropriate restriction enzyme and examined by Southern blot hybridization with the corresponding radiolabeled probe using standard methods, as previously described.<sup>13</sup> Immunoglobulin gene probes were as described by Korsmeyer et al<sup>14</sup>— $J_H$  (2.4-kilobase [kb] *sau3a* fragment),  $C_k$  (2.5-kb *EcoRI* fragment),  $C_\lambda$  (0.8-kb *BamHI-HindIII* fragment). T cell receptor gene probes were  $C_\beta$  (0.6-kb *BglII-EcoRV* fragment of YT35)<sup>15</sup> and  $C_\alpha$  (1.2-kb *EcoRI* fragment of clone V7d).<sup>16</sup>

DNA was also probed for sequences related to EBV, cytomegalovirus, and human T cell leukemia virus, types I, II, III, as well as for rearrangement of the *c-myc* oncogene.<sup>3,16,17</sup> The ontogeny of the tumor was studied by immunofluorescent staining of tissue sections using specific antisera to the immunoglobulin kappa and lambda light chains.

### RESULTS

Autopsy revealed extensive replacement of lymph nodes by large cells with vesicular nuclei and nucleoli. Masses containing similar cell types were distributed throughout the gastrointestinal tract, liver, and spleen. The brain showed generalized loss of gray and white matter with inclusion bodies consistent with cytomegalovirus. No lymphoma was found in the brain. On light microscopy, the histology of the tumor was consistent with a diffuse large-cell lymphoma. Immunofluorescent studies for immunoglobulin light chains revealed a predominance of lambda light chains, suggesting a monoclonal origin. Immunoglobulin gene studies performed on DNA prepared from the lymphoma (Fig 1)

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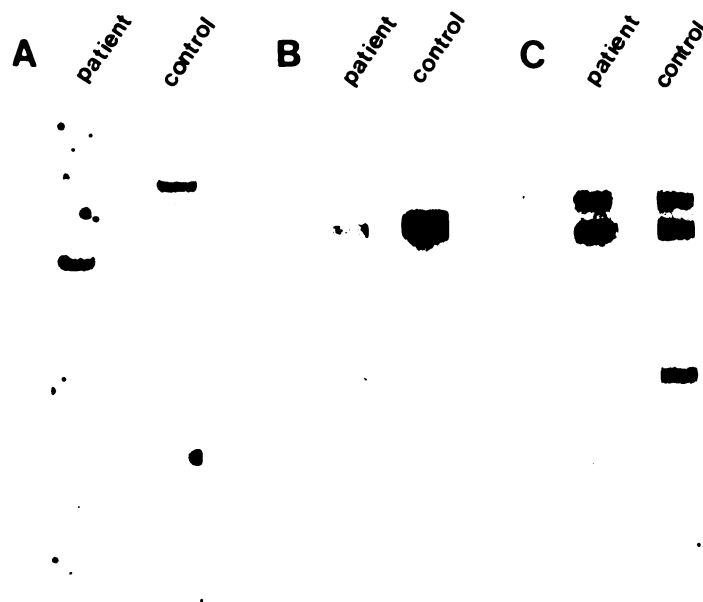
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**Fig 1.** Detection of immunoglobulin gene rearrangement by Southern hybridization. DNA from a biopsy specimen of the patient's lymphoma was examined by Southern blot hybridization using immunoglobulin gene probes as described in Materials and Methods. In all three panels (A through C) the lymphoma DNA is shown in the first lane, with control DNA (from a normal unrelated individual) in the germline configuration in the second lane. Dashes indicate the expected position of germline bands.<sup>14</sup> (A) Immunoglobulin heavy chain locus (*Bam*HI/*J*<sub>H2</sub> probe). A prominent rearranged band is evident at the lower arrowhead, consistent with a monoclonal B cell population. The absence of a second band of equal intensity suggests deletion of the second heavy chain locus in these cells. The faint persistent germline band may be due to a small amount of residual normal tissue in the biopsy specimen. The upper arrowhead marks a second faint rearranged band. This may represent incomplete detection of a rearrangement at the second heavy chain locus. We cannot exclude a rearrangement due to a second, smaller monoclonal cell population (a "biclinal" lymphoma). (B) Kappa light chain locus (*Bam*HI/*C*<sub>K</sub>). Only a faint germline band is evident in the lymphoma DNA. This is most consistent with deletion of both kappa gene loci, with the faint band due to residual normal tissue in the biopsy specimen, as above. (C) Lambda light chain focus (*Eco*RI/*C*<sub>λ</sub>). The absence of one germline band from the lymphoma DNA is evident. This pattern could be consistent with deletion or rearrangement of one lambda light chain gene. However, since nonlymphoid DNA from this patient was not examined, we cannot exclude the presence of a restriction fragment length polymorphism at the lambda light chain locus<sup>14</sup> as the explanation for this pattern.

revealed evidence for rearrangement at the heavy chain locus and probable deletion of both kappa light chain loci. Examination of the T cell receptor alpha and beta chain loci revealed exclusively the germline configuration with no evidence for clonal rearrangement (data not shown). This pattern is most consistent with monoclonal proliferation of B cell origin and of lambda light chain type. These results were in agreement with the immunofluorescent studies.

Serologic studies indicated antibodies to EBV early, nuclear, and capsid antigens, cytomegalovirus, and HTLV-III (Table 1). Antibodies to HTLV types I or II were not present. The tumor tissue expressed EBV nuclear antigen detected by immunofluorescence. Southern analysis revealed sequences related to EBV in the B cell lymphoma (Fig 2) but no hybridizing sequences using probes for cytomegalovirus, HTLV-III, HTLV-I, and HTLV-II. Rearrangement near the c-myc gene was also detected by Southern analysis (Fig 3).

#### DISCUSSION

B cell lymphomas occur with increased frequency in a variety of hosts with inherited or acquired deficiencies of the cellular immune system.<sup>18-21</sup> Considerable epidemiologic and

molecular biologic data suggest that some of these B cell lymphomas are associated with the presence of circular EBV genome and possibly integration of EBV into host genome.<sup>11,19</sup> Polyclonal B cell hyperplasia is frequently found in lymph nodes of patients with AIDS or AIDS-related complex.<sup>8</sup> In some cases, there appears to be an initial

**Table 1. Laboratory Studies**

Serology	
EBV-EA	1:80
EBV-VCA	1:640
EBNA	1:160
CMV-CF	1:128
HTLV-III-ELISA	+
HTLV-III-Western blot	+
Molecular Studies on Lymphoma DNA	
EBV	+
HTLV-III	-
Immunoglobulin rearrangement	+
T cell antigen receptor rearrangement	-

EBV, Epstein-Barr virus; EA, early antigen; VCA, viral capsid antigen; EBNA, EBV-nuclear antigen; CMV-CF, cytomegalovirus complement fixation; HTLV-III-ELISA, enzyme-linked immunosorbent assays.



**Fig 2.** Detection of EBV DNA in lymphoma tissue by Southern hybridization. DNA (20  $\mu$ g) extracted from lymphoma tissue obtained at autopsy was cleaved with *Bam*HI, subjected to electrophoresis (1.5 V/cm, 18 hours) through a 0.75% agarose gel and transferred to a nitrocellulose sheet. This gel replica was hybridized to PKD-52, a *Bam*W fragment of EBV in pBR322 (a gift from E. Kieff, University of Chicago, Chicago). Lane 1, 20  $\mu$ g lymphoma DNA, lane 2, 30 pg pBR322 was added to 20  $\mu$ g lymphocyte DNA from an EBV-seronegative control, equivalent to ten copies of viral DNA per cell. The hybridization in lane 2 is with pBR322 and not with EBV, as shown by different location of the hybridization band compared with lane 1.

polyclonal activation of B cells, followed by emergence of a monoclonal neoplasm. HTLV-III is the primary etiologic agent in AIDS.<sup>1-3</sup> The T4 antigen, or a closely related membrane antigen, appears to be necessary for entry and permissive infection of HTLV-III in T lymphocytes.<sup>22,23</sup> Because some activated B cells express the T4 antigen and, in vitro, HTLV-III can infect T4-bearing lymphoblastoid cell lines transformed by EBV, it is possible that HTLV-III could directly infect in vivo activated B cells and play a primary pathogenetic role in the development of B cell neoplasia in AIDS.<sup>23</sup> Our study of this case argues against such a model. There were no sequences related to HTLV-III in the B cell lymphoma, despite clear serologic evidence of HTLV-III infection in the host. A more likely model, based on our study, would be the emergence of an EBV-transformed B cell clone resulting from impaired cellular "immunologic surveillance" due to infection of T cells by HTLV-III.

Serologic studies in AIDS demonstrate high titers of antibodies to EBV antigens, suggesting reactivation of this virus in some patients.<sup>5</sup> Previous case reports have indicated



**Fig 3.** Detection of rearranged *c-myc* gene by Southern hybridization. Lymphoma tissue DNA (20  $\mu$ g) was cleaved with *Eco*RI, the DNA fragments were separated by electrophoresis (1.7 V/cm, 18 hours) through a 0.75% agarose gel. The DNA was then transferred to cellulose nitrate under partial vacuum, fixed, and hybridized with <sup>32</sup>P-labeled 3rd exon of human *c-myc* (Hu 3' *myc*, 1.2 kb, RI-*Cla*, 3rd exon) in pBR327 (Taub) (a gift from P. Leder, Harvard Medical School, Boston). Lane 1, Raji (Burkitt's lymphoma with detectable rearrangement of *c-myc*) cell DNA; lane 2, P3HR1 (Burkitt's lymphoma without detectable rearrangement of *c-myc*) cell DNA; lane 3, lymph node DNA from a fatal EBV infection in a patient with X-linked lymphoproliferative syndrome with a polyclonal, karyotypically normal EBV-induced B cell proliferation; lane 4, lymphoma tissue DNA from the AIDS patient.

chromosomal translocations t(8:14) and t(8:22) in B cell lymphoma in AIDS.<sup>6,7</sup> Such translocations occur in endemic (African) and nonendemic Burkitt's lymphoma.<sup>24-26</sup> Karyotype was not done in our case, but DNA rearrangement near the *c-myc* gene was detected. The *c-myc* oncogene is located on chromosome 8 and is rearranged in Burkitt's lymphoma unrelated to AIDS.<sup>7</sup> The pathogenesis of some B cell lymphoma in AIDS may involve initial polyclonal expansion of the B cell population due to reactivation of EBV with subsequent emergence of a transformed monoclonal population characterized by chromosomal rearrangement and disordered regulation of the *c-myc* oncogene. The emergence of the transformed clone may be facilitated by HTLV-III infection and impaired immune surveillance.

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