**Association of Epstein-Barr Virus with an Angioimmunoblastic Lymphadenopathy-Like Lymphoproliferative Syndrome**

A. CLINTON WHITE, Jr., M.D.,a,b BEN Z. KATZ, M.D.,b,c AND JEROME A. SILBERT, M.D.d

Infectious Disease Sections, aDepartment of Medicine, cDepartment of Pediatrics; bDepartment of Epidemiology and Public Health; dDepartments of Pathology and Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut

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A patient presented with fever, night sweats, generalized lymphadenopathy, cold agglutinin hemolytic anemia, and thrombocytopenia. Lymph node biopsy revealed a lymphoplasmacytic infiltrate sparing the cortical sinuses but associated with arborizing blood vessels. Epstein-Barr virus (EBV) DNA was demonstrated in tissue from an involved lymph node. These data suggest an association of EBV with a lymphoproliferative process that resembles angioimmunoblastic lymphadenopathy with dysproteinemia.

**INTRODUCTION**

Since its discovery in cultured lymphoblasts from a patient with Burkitt’s lymphoma [1], Epstein-Barr virus (EBV) has been linked to a number of lymphoproliferative syndromes [2,3]. EBV shows a tropism for nasopharyngeal epithelial cells and B lymphocytes, the latter becoming immortalized when infected. Infectious mononucleosis, the most common clinical manifestation of acute EBV infection, is associated with polyclonal B-cell proliferation, which is evidently controlled by cytotoxic T cells, natural killer cells, and neutralizing antibodies. In immunocompromised hosts, however (e.g., patients with the X-linked lymphoproliferative syndrome, organ transplant recipients, and patients with human immunodeficiency virus infections), EBV infection may progress to fatal lymphoproliferation or lymphoma [2,3].

In the early 1970s, several groups described a lymphoproliferative syndrome in elderly patients, consisting of generalized lymphadenopathy, fever, hepatosplenomegaly, skin rash, malaise, Coombs-positive hemolytic anemia and other hematologic syndromes, and polyclonal hypergammaglobulinemia; this syndrome is usually referred to as angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) or immunoblastic lymphadenopathy [4–6]. Several groups have subsequently described patients whose clinical presentation resembles AILD but who lack some of its histological features; this syndrome is usually termed autoimmune-associated lymphadenopathy [7–9]. The etiology of AILD and related syndromes remains unclear [4–6,10,11].

**Abbreviations**: AILD: angioimmunoblastic lymphadenopathy  CMV: cytomegalovirus  EA: early antigen  EBNA: Epstein-Barr virus nuclear antigen  EBV: Epstein-Barr virus  VCA: viral capsid antigen

Address reprint requests to: Ben Z. Katz, M.D., Dept. of Pediatrics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

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We report a patient who presented with clinical and histologic features suggestive of AILD (though lacking the classic histologic features), who had EBV genome detected by Southern blot analysis in a pathologically involved lymph node.

CASE REPORT

The patient was a 61-year-old white man who presented in October 1985 to the West Haven Veterans Administration Medical Center with a four-week history of lethargy, malaise, chills, fever, night sweats, and abdominal pain. On physical examination, he had a temperature of 102°F, generalized lymphadenopathy, and a palpable spleen. Laboratory evaluation included a hemoglobin of 5.9 g/dL, hematocrit of 14.2 percent, platelet count of 96,000/mm³, and a white cell count of 12,500/mm³ with 1 percent band forms, 72 percent polymorphonuclear leukocytes, 10 percent lymphocytes, 15 percent monocytes, 2 percent atypical lymphocytes, and a reticulocyte count of <0.1 percent. The peripheral blood smear was remarkable for plasmacytoid lymphocytes and rouleaux formation. Total protein was 7.2 g/dL, albumin 3.6 g/dL. Serum protein electrophoresis showed multiple faint bands extending from the beta through the gamma regions. A strongly reacting, low-titer IgM cold agglutinin was present, which reacted equally with adult and umbilical cord cells. Skin tests showed the patient to be anergic to candida and mumps. Abdominal and chest CT scans confirmed the presence of generalized lymphadenopathy and splenomegaly. Bone marrow aspirate and biopsy showed hypercellularity, focal fibrosis, and increased numbers of large mononuclear cells. A left supraclavicular lymph node biopsy revealed almost total effacement of the normal architecture by a lymphoplasmacytic infiltrate composed of cells with a coarse and blotchy chromatin pattern, rare mitoses, and a few eccentric nuclei; the infiltrate stained strongly positive with methyl green pyronine. Immunohistochemical stains showed staining with antibody to kappa and lambda light chains as well as to T-cell markers. The patient was transfused and begun on prednisone, 100 mg per day, in an attempt to control the anemia and thrombocytopenia. The patient’s anemia, thrombocytopenia, and fevers slowly resolved. After four weeks of therapy, the dose of prednisone was tapered. He again developed fever, night sweats, and abdominal pain.

Eight weeks following his initial admission, the patient was readmitted for evaluation of hematochezia; at this time, his prednisone was stopped. On the third hospital day, he developed epistaxis and fever. His hemoglobin was 11.6 g/dL, hematocrit 35.6 percent, platelet count 2,000/mm³ (down from 166,000 six days earlier), and reticulocyte count 4 percent. Cold agglutinins were negative; direct Coomb’s test was positive for anti-C3. He was started on high-dose methylprednisolone in an attempt to control his thrombocytopenia and on mezlocillin and gentamicin empirically. On this regime, he developed a macular rash, which resolved when the antibiotics were discontinued. Despite high doses of steroids, daily fevers, hemolysis, and thrombocytopenia persisted. A right axillary lymph node biopsy revealed complete effacement of the node and infiltration of the perinodal fat by lymphoplasmacytic cells, similar to that seen in the previous lymph node biopsy. There was minimal involvement of the cortical sinuses. Proliferation of arborizing blood vessels was noted, but necrosis and eosinophilic material were not present. Immunofluorescence staining of cells from the node showed that 63 percent were Leu 4 positive, 16 percent Leu 3 positive, 41 percent Leu 2 positive (T helper: T suppressor, 0.39:1), 36 percent B1 positive, 25 percent positive for anti-lambda, and 25 percent positive for anti-kappa. In January of 1986, the patient underwent splenectomy, exploratory laparotomy, abdominal lymph node
biopsy, and liver biopsy. The spleen showed congestion and a polymorphic infiltrate. The liver was normal. Routine cultures of lymph node tissue for bacteria, mycobacteria, fungi, and viruses were negative. Over the next ten weeks, as the prednisone was gradually discontinued, fever, hemolytic anemia, and thrombocytopenia resolved. Except for lymphadenopathy, which has continued to wax and wane, he has remained well throughout 22 months of follow-up.

METHODS

**EBV Serology**

Antibodies to EBV capsid (VCA), early (EA), and nuclear (EBNA) antigens were detected by standard immunofluorescence techniques [12].

**Immunofixation Electrophoresis**

Immunofixation electrophoresis was performed as previously described [13] on samples from the patient’s initial admission. Samples included serum as well as cold agglutinins, which were eluted from the patient’s red cells with warm saline after washing the cells with cold saline.

**Detection of EBV DNA**

DNA was extracted from the patient’s abdominal lymph node biopsy and probed, essentially as previously described. Briefly, approximately two grams of the patient’s lymph node was sliced and shredded with a razor blade, suspended in phosphate buffered saline without Ca\(^{2+}\) or Mg\(^{2+}\), and put through 15 strokes of a homogenizer. The homogenate was digested first with pronase and sodium dodecyl sulfate and then with RNase, with a final precipitation in 95 percent ethanol [14]. Approximately three micrograms of the resulting cellular DNA was then digested with the restriction endonuclease BamHI, electrophoresed on a 0.5 percent agarose gel, and transferred to nitrocellulose, using Southern’s method [15]. EBV DNA sequences were detected by probing with the *EcoRI A* DNA fragment of the standard EBV strain FF41 in plasmid pACYC, labeled with \([^{32}P]\)dCTP by nick translation [14].

RESULTS

Immunofixation electrophoresis showed at least five distinct IgM bands, with both IgM-lambda and IgM-kappa bands. Immunofixation electrophoresis of the cold agglutinins also revealed IgM with both lambda and kappa light chains.

Viral serologies are presented in Table 1. The patient shows evidence of previous EBV infection, as determined by the presence of titers to both VCA and EBNA and the absence of either IgM antibody to VCA or a rising IgG VCA antibody titer.

DNA extracted from the patient’s abdominal lymph node was positive for EBV DNA by Southern blot analysis (see Fig. 1). The strongest hybridization was to the *BamHI W* fragment, which is to be expected since it is a repeated element in the EBV genome. The usual hybridization to the other *BamHI* fragments (C, F, and H) within *EcoRI A* was also seen. (*BamHI* fragment Q is often not observed even though it is within *EcoRI A* because it is so close in molecular weight to the repeated *BamHI W* fragment; *BamHI Y* does not always resolve well, because it is near the limit of resolution of a 0.5 percent agarose gel. *BamHI H* commonly varies in size between
TABLE 1
Viral Titers

| Antigen       | Admission 10/85 | 12/85 | Abdominal Surgery 1/86 | 3/86 |
|---------------|----------------|-------|------------------------|------|
| EBV VCA       |                |       |                        |      |
| IgG           | 1:320          | 1:640 | 1:640                  | 1:320|
| IgM           | <1:10          | <1:10 | <1:10                  |      |
| EA restricted | 1:20           | 1:40  | 1:40                   | 1:20 |
| EA diffuse    | 1:5            | 1:5   | <1:5                   | 1:5  |
| EBNA          | 1:20           | 1:40  | 1:20                   | 1:40 |
| CMV           | 1:32           | 1:32  | 1:32                   |      |
| HIV           | Negative       |       |                        |      |

Serologies to Epstein-Barr virus VCA, EA, and EBNA as well as to cytomegalovirus (CMV) were measured with standard immunofluorescence techniques. HIV titer was measured by ELISA.

different isolates of EBV, as it does here between the control and the patient's specimen [16]).

DISCUSSION

The etiology of AILD and related syndromes is unknown. The development of this family of disorders has variously been associated with allergens, toxins, and infectious agents; however, whether one of these actually triggers the onset of the disease is unknown. (For a recent review, see [17]). The histologic picture is suggestive of a reactive process, composed predominantly of T lymphocytes. Recent studies suggest

FIG. 1. Detection of EBV DNA in the patient's lymph node. Approximately three μg of total cellular DNA from the patient's lymph node (specimen) or 15 μg of control DNA (from a known EBV-positive lymphoblastoid cell line) were digested with the restriction enzyme BamHI and probed with a nick-translated, [32P]-labeled plasmid containing the EcoRI A region from a standard laboratory strain of EBV. The BamHI fragments resulting from the digestion are shown at the left of the figure; for further details, see text.
EBV AND ANGIOIMMUNOBLASTIC LYMPHADENOPATHY

that, when examined by probes for rearranged immunoglobulin and T-cell receptor genes, clonal expansion of T and/or B cells may be seen [18–20]. Because of these data, and the fact that up to 20 percent of cases can progress to lymphoma, there has been much speculation that this group of syndromes represents a pre-lymphomatous state [17,18,21].

In our case, while there was the suggestion of oligoclonal bands on serum protein electrophoresis and immunofixation electrophoresis, no evidence of a prominent clonal lymphoproliferation was present in lymph node tissue when examined by immunohistochemical and immunofluorescence staining of lymph node cells. EBV genome was demonstrated, however, despite the fact that the patient did not have serologic evidence of acute or reactivation infection.

Previous studies have demonstrated EBV DNA and EBNA in lymphoid tumors from patients with organ transplants, AIDS, and other immunodeficiencies, in the central nervous system, and African Burkitt's lymphoma, as well as in epithelial cells from patients with nasopharyngeal carcinoma, and, recently, in T-cell lymphomas from patients with severe, chronic EBV infections [1,2,3,14,22–28]. In contrast, studies using Southern blotting have only rarely demonstrated the presence of EBV DNA in a large number of other lymphoproliferative processes, even in the immunosuppressed [14]. These EBV-associated lesions are usually but not invariably accompanied by serologic evidence of EBV reactivation in the affected individual; in fact, EBV serologic responses may be abnormal in immunocompromised patients, even in the face of a progressive EBV infection [29]. Thus, a role for EBV in the pathogenesis of this patient's lymphoproliferative process cannot be excluded on the basis of serologic data.

Bornkamm et al. described one case of immunoblastic lymphadenopathy in which EBV DNA was demonstrated by reassociation kinetics [30]. Virelizier et al. described a child with presumed AILD who had persistently elevated titers to EBV antigens in serum and EBNA in affected lymph node cells [17,31]. We now report an additional association of EBV DNA, as detected by Southern blot analysis, with an AILD-like syndrome. Taken together, these reports suggest an association between EBV and a small number of lymphoproliferative syndromes associated with autoimmunity, such as AILD.

What role EBV or any other associated agent plays in the pathogenesis of AILD or AILD-like illness is, at present, unclear. The increased number of suppressor T cells in the involved lymph node may have been a reaction to virally infected B cells. It is also conceivable, however, that EBV may have infected the patient's T cells, as has been described for some T-cell lymphomas in patients with severe, chronic EBV infections [27,28].

Studies of the nature of the EBV infection associated with various lymphoproliferative disorders may further understanding of the pathogenesis of these diseases. In addition, more sensitive genomic analysis of biopsied material, especially the use of the polymerase chain reaction, will probably add to the expanding spectrum of EBV-associated disorders.

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EBV AND ANGIOIMMUNOBLASTIC LYMPHADENOPATHY

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