We describe a patient with angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) who subsequently developed large-cell immunoblastic lymphoma of B-cell immunophenotype. At the time of the initial diagnosis, histologic examination of an inguinal lymph node showed typical features of AILD, and there was no evidence of a monoclonal B-cell population by immunohistochecmical analysis. In situ hybridization and Southern blot analysis for Epstein-Barr virus (EBV) were negative. At autopsy 2 years later, the patient had widespread lymph node and organ involvement by large-cell immunoblastic lymphoma of B-cell immunophenotype. Southern blot analysis performed on DNA extracted from lymph nodes, liver, and spleen showed two patterns of Ig heavy chain and light chain gene rearrangements. The T-cell receptor β chain gene was in the germline configuration. Analysis with an EBV terminal repeat region probe showed two clonal populations that paralleled the Ig gene rearrangement studies. Double-labeling immunohistochemistry and in situ hybridization confirmed the presence of EBV within the neoplastic B cells. The data support the hypothesis that EBV was not etiologically related to AILD in this case, and that EBV proliferation may occur after the onset of the disease. Further, the data suggest that some B-cell lymphomas that arise in the setting of AILD resemble EBV-associated B-cell lymphomas that arise in other immunodeficiency states.

**CASE REPORT**

A 46-year-old man presented in October 1986 to a local community hospital with cough, generalized pruritic rash, and lymphadenopathy of several weeks’ duration. A supraclavicular lymph node biopsy specimen was interpreted as “diffuse histiocytic lymphoma.” The patient had fever, night sweats, weight loss, anemia, and polyclonal hypergammaglobulinemia. Histologically, the lymph node architecture is effaced by a polymorphous cellular infiltrate composed of lymphocytes, plasma cells, eosinophils, histiocytes, and immunoblasts. Finely arborizing blood vessels course through the lymph node, and intercellular deposits of amorphous, eosinophilic material may be present. The majority of lymphoid cells are proliferating T cells, admixed with smaller numbers of polyclonal B cells. Progression to malignant lymphoma is marked by the presence of clusters or sheets of large lymphoid cells or immunoblasts. Gene rearrangement studies have been performed to assess the clonality of AILD and malignant lymphomas arising in patients with AILD. Most studies have shown rearrangements of the T-cell receptor (TCR) genes, although others have also shown rearrangements of the Ig genes. Lipford et al have shown TCR and Ig gene rearrangements in the peripheral blood and lymph nodes of patients with AILD that appeared and regressed over time. They proposed that AILD represents a premalignant disorder of altered immunoregulation characterized by clonal expansions of B and T cells that result from decreased immunocompetence. Thus, despite the frequent presence of clonal lymphoid populations, it is still unclear whether AILD is a benign but clonal immunoproliferative disorder or malignant lymphoma at its inception. Regardless, AILD is associated with profound immunologic deficits. Patients are plagued by frequent infections, even in the absence of therapy-induced cytopenias.

Recent studies using the polymerase chain reaction (PCR) and in situ hybridization have shown Epstein-Barr virus (EBV) DNA or RNA in lymph node biopsies from patients with AILD. This finding has led some investigators to propose a direct role for EBV in the pathogenesis of AILD. Alternatively, the presence of large amounts of EBV in these specimens may represent an epiphenomenon that reflects the profound immunosuppression of patients with this disease. In support of this view, Weiss et al have recently shown that the virus resides primarily within B lymphocytes in the AILD lymph node, and that the majority of the T cells in AILD and AILD-like lymphomas do not contain the virus.

In this report, we describe a patient with AILD who subsequently developed an aggressive B-cell malignant lymphoma. EBV was detected in the lymphoma, but not in the previously biopsied lymph node that showed AILD. The lymphoma was bicaloncal, based on the results of gene rearrangement studies that correlated with the presence of two clonal episomal populations of EBV. The data in this unusual case suggest that the profound immunosuppression associated with AILD, and possibly the additional immunosuppression secondary to chemotherapy, predisposed this patient to either a primary or reactivated EBV infection. The findings argue against the hypothesis that EBV was the etiologic agent of AILD. Similar to malignant lymphomas that arise in other immunodeficiency states, EBV infection in AILD may lead to polyclonal and occasionally oligoclonal or monoclonal B-cell expansions that eventuate in lymphoma.

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The patient developed superior vena cava syndrome, received radiation therapy and corticosteroids, and was referred to the Clinical Center at the National Institutes of Health (NIH).

The patient was seen at the NIH in January 1987. Physical examination showed an ill-appearing man with axillary, epitrochlear, and inguinal lymphadenopathy, and a morbiliform rash that spared only his feet. The patient also had cough, fevers, and a 30-lb weight loss. Past medical history, social history, and family history were unremarkable. Laboratory studies included a hemoglobin of 115 g/L; hematocrit of 0.33; white blood cell count (WBC) of 4.4 \times 10^9/L; with a normal differential count; platelet count of 233 \times 10^9/L; and an erythrocyte sedimentation rate of 65 mm/h. Serum IgA was slightly elevated at 4.81 g/L; serum IgG of 9.72 g/L and IgM of 1.06 g/L were normal. All other laboratory studies were normal. The chest radiograph showed a widened mediastinum. Review of the supraclavicular lymph node biopsy performed previously and biopsy of a left inguinal lymph node performed at the NIH were diagnostic of AILD. Bone marrow biopsy and aspirate smear were consistent with involvement by AILD.

The patient was begun on a 12-month course of intravenous cyclophosphamide and corticosteroids, and he rapidly achieved complete clinical remission. He was well until May 1988, 3 months after completing chemotherapy, when he developed cough, fevers, rash, and generalized lymphadenopathy. Monthly chemotherapy was re instituted, and clinical remission was achieved for an additional 4 months. Subsequently, from September until his death in December 1988, the patient's course was complicated by Pneumocystis carinii pneumonia, Haemophilus influenzae pneumonia, steroid myopathy, deep vein thrombosis, Candida esophagitis, and a generalized macular erythematous rash. Two skin biopsies were performed. A punch biopsy from the back showed involvement by AILD, with an increased number of immunoblasts. A second skin biopsy from the wrist studied immunophenotypically showed an atypical lymphoid infiltrate consistent with malignant lymphoma, large-cell immunoblastic type, of B-cell immunophenotype. The patient died of multiorgan failure. The autopsy showed generalized lymphadenopathy with massive mediastinal and abdominal lymphadenopathy, hepatosplenomegaly, and lymphomatous infiltrates involving the lungs, skin, bone marrow, gastrointestinal tract, kidneys, testes, and central nervous system.

**MATERIALS AND METHODS**

**Histologic and immunophenotypic analysis.** Portions of tissue obtained at biopsy or autopsy were fixed in B5 fixative or 10% formalin. Fixed, paraffin-embedded sections were stained with hematoxylin and eosin. Immunophenotypic studies were performed on fresh tissue using either cell suspensions analyzed by flow cytometry or frozen sections analyzed by the avidin-biotin complex (ABC) immunoperoxidase technique, as described previously. The panel of antibodies used included Ig heavy (\(\gamma_1, \gamma_2, \gamma_3, \delta\)) and light (\(\kappa, \lambda\)) chains (Tago Inc, Burlingame, CA). B4 (CD20), CT11 (CD22), My4 (CD14), T6 (CD1), (Coulter, Hialeah, FL), Leu14 (CD22), Leu1 (CD5), Leu2a (CD8), Leu3a (CD4), Leu4 (CD3), LeuM5 (CD1c), LeuM1 (CD15) (Becton Dickinson, San Jose, CA), and 3A1 (CD7) (courtesy of B.F. Haynes, MD).

**Southern blot analysis.** High molecular weight genomic DNA was extracted from lymph nodes, spleen, liver, and peripheral blood leukocytes using a standard proteinase K/RNase phenol-chloroform technique. DNA (10 to 15 mg) was digested with EcoRI, HindIII, and BamHI restriction endonucleases (Bethesda Research Laboratories, Gaithersburg, MD). Digested DNA was separated by electrophoresis on 0.7% agarose gels, depurinated with 0.25 N HCl, and transferred in 0.4 N NaOH onto nylon membranes as previously described. The membranes were hybridized with \(^{32}P\)-labeled DNA probes. The TCR \(\beta\) chain gene was assessed with a 400-bp cDNA probe specific for the constant region (CH). All other probes were genomic. The Ig heavy chain gene and the \(\kappa\) light chain gene were assessed with probes derived from the joining regions, JH and JK, respectively. Rearrangements were confirmed by the use of at least two restriction endonucleases.

DNA digested with BamHI was also probed with the Xho I 1.9-kb genomic fragment derived from the terminal repeat region of EBV. Probes derived from this region of EBV can be used to assess clonality as well as the replicative status of the virus. In latently infected B cells, viral DNA is present in circular episomal form, and different epimorphs have different numbers of terminal repeated segments. If only one species of EBV DNA is present in a latently infected cell population, a single restriction fragment of greater than 8 kb is observed in DNA digested with BamHI. In latently infected oligoclonal lesions, more than one restriction fragment will be present. During replication, episomal DNA is cleaved randomly between the terminal repeated sequences, generating a ladder of 4- to 6-kb fragments that differ from each other by 500-bp increments.

**RESULTS**

**Pathologic and immunophenotypic findings.** The 1987 left inguinal lymph node biopsy specimen obtained at the NIH was 1.8 \(\times\) 1.5 \(\times\) 1.0 cm, with a homogeneous, tan cut surface. Histologically, the 1986 supraclavicular lymph node biopsy obtained elsewhere and the 1987 lymph node biopsy were similar. In both biopsy specimens the lymph node architecture was effaced by a polymorphous infiltrate of small lymphocytes, plasma cells, eosinophils, and scattered immunoblasts (Fig 1A). Finely arborizing blood vessels were prominent throughout the lymph node. Normal follicles were absent, but a few follicular remnants were identified. A cell suspension obtained from the 1987 specimen analyzed by flow cytometry showed that approximately 60% of the cells were normal T cells with a CD4/CD8 ratio of 1:1. Approximately 15% of the cells were polytypic B cells. The remaining 25% of cells were a mixture of histiocytes and granulocytes. A bone marrow biopsy showed focal fibrosis of the medullary space associated with plasma cells, eosinophils, and immunoblasts, interpreted as consistent with involvement by AILD.

A punch biopsy of skin from the back performed in November 1988, 3 weeks before the patient's death, contained a dermal perivascular and perivascular lymphoid infiltrate consistent with AILD with an increased number of immunoblasts. One week later, immunophenotypic studies performed on a punch biopsy of skin from the wrist showed an atypical lymphoid infiltrate consistent with malignant lymphoma, large-cell immunoblastic type, of B-cell immunophenotype. The neoplastic cells expressed pan-B-cell antigens (CD19, CD20, CD22) and IgG, but were negative

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Fig 1. Lymph nodes involved by AILD and malignant lymphoma. (A) AILD involving the 1987 left inguinal lymph node. The normal architecture was effaced by a polymorphous infiltrate of small lymphocytes, plasma cells, eosinophils, and occasional immunoblasts. Finely arborizing blood vessels were present (hematoxylin and eosin, original magnification × 630). (B) Malignant lymphoma, large-cell immunoblastic type (Working Formulation), involving a periaortic lymph node obtained at autopsy. Large, multilobated lymphoid cells with prominent karyorrhexis replaced normal lymph node (hematoxylin and eosin, original magnification × 630).

for other Ig heavy chains, as well as T-cell and histiocyte-associated markers. Ig light chain staining was technically suboptimal and uninterpretable.

Autopsy showed generalized lymphadenopathy and widespread organ involvement. On gross examination, many lymph nodes contained foci of hemorrhage and necrosis. Microscopically, the architecture of all lymph nodes was effaced by a monomorphic population of malignant lymphoid cells with large, multilobated nuclei, prominent nucleoli, and conspicuous eosinophilic cytoplasm (Fig 1B). The mitotic rate was brisk and karyorrhexis was extensive. Using the Working Formulation, the lymphoma was classified as large-cell immunoblastic. The splenic white pulp was replaced by lymphoma that extended into the red pulp. The lymphoma extensively infiltrated the portal tracts of the liver. Other sites involved by lymphoma included the skin, bone marrow, kidney, testes, gastrointestinal tract, and central nervous system.

Southern blot analysis. Southern blot analysis for antigen receptor gene rearrangements was performed on DNA prepared from an antemortem peripheral blood leukapheresis sample obtained before the development of overt lymphoma, and from multiple lymph nodes (right and left axillary, paracaval, and two periaortic), liver, and spleen obtained at autopsy. Tissue from the 1987 inguinal lymph node biopsy involved by AILD was limited; DNA was sufficient only for one BamHI digest.

All autopsy tissue samples contained clonal Ig heavy and light chain gene rearrangements. Two patterns of rearrangements were detected, depending on the site examined. Figure 2A shows the rearrangement patterns observed in HindIII-digested DNA hybridized with the JH probe. Five of the specimens, including the left axillary lymph node (lane 4), one of two periaortic lymph nodes (lane 7), liver (lane 8), and spleen (lane 9) displayed two major rearrangements (JH2 and JH3), in addition to the germline band. A second pattern was observed in the right axillary (lane 3) and paracaval lymph nodes (lane 5). In addition to the common JH2 and JH3, two other major bands of rearrangement were observed, JH1 and JH4. In a second periaortic lymph node (lane 6), the rearranged bands were faint, but JH1 and JH4 bands also may be present. Figure 2B shows the same blot rehybridized with a probe for the Ig κ light chain joining region. Again, two patterns of rearrangement.
mens obtained from all sites contained a major identical episomal band (E1). A second major episomal band (E2) was found in samples from right axillary (lane 3) and paracaval lymph nodes (lane 5). E2 is present, but at low intensity, in the left axillary (lane 4) and periaortic lymph nodes (lanes 6 and 7), and is completely absent from the liver (lane 8) and spleen (lane 9). Thus, E1 appears to correspond to the first clone, and E2 appears to correspond to the second clone detected by Ig gene rearrangement. Because the amount of the second clonal episomal population (E2) is small in the left axillary (lane 3), first periaortic (lane 6), and second periaortic lymph nodes (lane 7), the corresponding Ig gene rearrangements are either faint (first periaortic lymph node) or undetectable (left axillary and second periaortic lymph nodes). The higher sensitivity of the EBV hybridization is due to the presence of multiple episomes that exist in the majority of EBV-infected neoplasms compared with the single-copy Ig gene.

All of the lymph nodes obtained at autopsy, but not liver or spleen, also contained replicating virus, as indicated by the ladder of low molecular weight bands that correspond to linear replicating forms.13 Low molecular weight bands were easily seen in specimens obtained from the left axillary were seen. Each site showed one common gene rearrangement, Jx2, as well as DNA in the germline configuration. However, analogous to the extra bands identified in the JH hybridization, the right axillary (lane 3) and paracaval lymph nodes (lane 5) contained an additional, identical rearrangement, Jx2. In the second periaortic lymph node (lane 6), the presence of a Jx2 band cannot be excluded, as its size is close to the germline fragment and the lane is overexposed. These findings suggest that the lymphoma is composed of two genetically distinct clones. The first clone, present in samples obtained from all sites, contributed two JH (JH2 and JH3) rearrangements and one Jx (Jx1) rearrangement. The second clone, easily detectable only in the right axillary and paracaval lymph nodes, contributed the additional JH (JH1 and JH4) and Jx (Jx2) gene rearrangements. The TCR \( \beta \) chain was in the germline configuration in all specimens, including *Bam*HI-digested DNA obtained from the left inguinal lymph node biopsy that was diagnostic of AILD (data not shown). DNA obtained from peripheral blood leukocytes at the time of diagnosis of AILD showed the germline configuration (Fig 2A and B) for all probes studied.

EBV DNA was identified in all specimens obtained at autopsy (Fig 3) using an EBV terminal repeat region probe. This probe can be used to determine the presence or absence of EBV, the number of episomes present, and the replicative status of the virus.12,13 The pattern of involvement in Fig 3 parallels the results obtained with the Ig gene probes. Specimens obtained from all sites contained a major identical episomal band (E1). A second major episomal band (E2) was found in samples from right axillary (lane 3) and paracaval lymph nodes (lane 5). E2 is present, but at low intensity, in the left axillary (lane 4) and periaortic lymph nodes (lanes 6 and 7), and is completely absent from the liver (lane 8) and spleen (lane 9). Thus, E1 appears to correspond to the first clone, and E2 appears to correspond to the second clone detected by Ig gene rearrangement. Because the amount of the second clonal episomal population (E2) is small in the left axillary (lane 3), first periaortic (lane 6), and second periaortic lymph nodes (lane 7), the corresponding Ig gene rearrangements are either faint (first periaortic lymph node) or undetectable (left axillary and second periaortic lymph nodes). The higher sensitivity of the EBV hybridization is due to the presence of multiple episomes that exist in the majority of EBV-infected neoplasms compared with the single-copy Ig gene.

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(lane 4) and periaortic lymph nodes (lanes 6 and 7), and were detectable in specimens obtained from the right axillary and paracaval lymph nodes on long exposures (data not shown). Interestingly, there was no evidence of EBV in the original biopsy diagnostic of AILD (lane 1) or in peripheral blood leukocytes (lane 2) collected at the time of diagnosis of AILD.

**EBV RNA in situ hybridization.** The biopsy specimen was diagnostic of AILD was found to be negative for EBV RNA by in situ hybridization (data not shown). The mRNA in this specimen was intact, as determined with a poly d(T) probe. In contrast, and consistent with the EBV Southern blot data, in situ hybridization performed on a postmortem paracaval lymph node identified EBV in the nuclei of the atypical cells (Fig 4). Double-labeling immunohistochemistry and in situ hybridization performed on this lymph node showed that the majority of EBV-positive cells were positive for the B-lineage marker L26 (CD20) and negative for the T-cell-associated antigen Leu22 (CD43), consistent with a B-cell immunophenotype (data not shown).

**DISCUSSION**

AILD is generally considered to be a lymphoproliferative disorder of T lymphocytes. The majority of the proliferating cells are T cells, and molecular studies of AILD have shown rearrangements of the TCR genes in the majority of cases. Occasional cases also contain Ig gene rearrangements. Patients with AILD often are profoundly immunosuppressed, and infection is a common cause of death. Another serious complication of AILD is the development of aggressive non-Hodgkin’s lymphomas, the majority of which are of T-cell phenotype. A smaller percentage of the lymphomas, approximately 15%, are of B-cell phenotype. Thus, as suggested previously by others, AILD may be considered a disorder of immune regulation in which clones of T and B cells proliferate, with a high probability of overt malignant transformation.

EBV has been found frequently in AILD and in lymphomas arising in the setting of AILD. For example, Weiss et al found EBV in 96% of cases of AILD or AILD-like lymphoma using FTR and in situ hybridization. As a result, some have suggested that EBV is the etiologic agent of AILD. However, others have proposed that EBV infection is a consequence of immunodeficiency, rather than the cause of the disease. The findings in this case are consistent with the latter hypothesis, because there was no evidence of EBV by Southern blot analysis or in situ hybridization in the left inguinal lymph node biopsy, which was diagnostic of AILD. However, we cannot exclude the presence of EBV at other sites, and serologic studies for EBV infection were not performed.

The findings in this case also raise the possibility that EBV plays a role in the pathogenesis of at least some B-cell lymphomas that arise in the setting of AILD. Numerous lymph nodes from multiple sites, liver, and spleen obtained at autopsy contained EBV, as shown by Southern blot analysis and/or in situ hybridization. These results appear to be analogous to B-cell lymphomas associated with other immunodeficiency states, such as infection by human immunodeficiency virus, organ transplantation, or congenital immunodeficiency. For example, virtually all of the lymphomas that arise in immunosuppressed allograft recipients have been shown to contain EBV. These patients fail to develop an effective EBV-specific cytotoxic T-cell response that normally limits EBV infection, and they also have abnormal humoral immune responses. Furthermore, in lymphomas that arise after transplantation, biopsy specimens from different anatomic sites in the same patient have been shown to have different patterns of Ig gene rearrangements and EBV. The findings presented in this report are similar. In multiple anatomic sites, at least two Ig heavy chain and κ light chain gene rearrangements were identified by Southern blot analysis, with different patterns of rearrangement depending on the anatomic site. The EBV terminal repeat region probe also showed two predominant clonal populations of episomal EBV DNA, as well as other minor clonal populations and replicating virus. The two predominant clones appeared to parallel the two clones detected by Ig gene rearrangement studies.

By what mechanism might B-cell lymphomas arise in the setting of AILD? Conceivably, the deficit in cellular immunity that accompanies AILD might permit primary EBV infection or reactivation of latent infection, followed by the
oligoclonal expansion of immortalized EBV-infected immunoblasts of B-cell phenotype. This is reflected in the proliferation of B immunoblasts and Ig gene rearrangements. Some EBV-infected cells may have an inherent growth advantage; these cells may eventually predominate. One or more clones may undergo a second genetic event, leading to the emergence of a malignant clone or clones\(^6\) manifested as malignant lymphoma.

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B-cell lymphoma after angioimmunoblastic lymphadenopathy: a case with oligoclonal gene rearrangements associated with Epstein-Barr virus

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