

Fatal Epstein-Barr Virus Infection in a Child With Acute Lymphoblastic Leukemia in Remission¹

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Abstract

A 9-year-old white boy developed a fatal primary Epstein-Barr virus (EBV) infection while receiving chemotherapy for acute lymphoblastic leukemia in remission. Histopathological findings at the height of the proliferative phase of the illness were compatible with a virally induced hemophagocytic syndrome. The infection spontaneously converted to complete aplasia of the bone marrow and lymph nodes. Serological studies disclosed that the patient had no antibodies to EBV prior to the infection, but during the acute phase he showed a spectrum and titers of antibodies to EBV-specific antigens characteristic of a current primary EBV infection. A lymph node biopsy obtained 5 weeks after onset revealed Epstein-Barr nuclear antigen in approximately 50% of the cells. The boy's condition deteriorated rapidly, with disseminated candidiasis resulting in cardiorespiratory failure and death. Lymph nodes obtained at autopsy no longer contained Epstein-Barr nuclear antigen-positive cells.

Introduction

Primary infections by EBV³ have been reported in a small number of children with ALL in remission. In these patients, the course of the infection did not appear to be influenced by either the underlying leukemia or chemotherapy (7, 12, 21).

In this report, we present a case of EBV infection in a boy with ALL in remission who was still receiving chemotherapy. During the course of the viral infection, the child developed a lymphoproliferative disorder that spontaneously converted to complete aplasia of the bone marrow and lymph nodes and resulted in a fatal infection by *Candida albicans*.

Case Summary

A 6-year-old white boy was referred to St. Jude Children's Research Hospital on July 28, 1976, for treatment of ALL. There was no family record of immunodeficiency, fatal infectious mononucleosis, or lymphomas. The boy had 2 healthy brothers and 2 maternal uncles who had died in infancy with congenital heart disease and pneumonia. At the time of diagnosis of ALL, he had a hemoglobin of 6.5 g/dl, a circulating WBC of 70,000/cu mm with 89% blasts, a normal chest

roentgenogram, and normal findings from a cerebrospinal fluid examination. A bone marrow examination confirmed the clinical impression of ALL. Bone marrow blasts did not form rosettes with sheep erythrocytes (11).

The patient was treated according to the Total Therapy IX Protocol Study, which specified induction of remission with prednisone, vincristine, daunorubicin, and asparaginase. This was followed by central nervous system preventive therapy with 2400 rads cranial irradiation and 5 doses of intrathecal methotrexate. Maintenance therapy consisted of daily p.o. 6-mercaptopurine and weekly i.v. doses of methotrexate. After 30 months of complete remission, before cessation of therapy, open testicular biopsies disclosed leukemia confined to the left testis, which had been suspected on clinical examination. He received radiation therapy (2400 rads) to the left testis and reinduction chemotherapy with prednisone and vincristine followed by 6-mercaptopurine daily and methotrexate i.v. weekly. Three months later, in June 1979, he developed an illness characterized by malaise and low-grade fever that resolved spontaneously over the next 3 weeks.

On October 29, 1979, when he was 9 years, 8 months old, the boy presented again with a 5-day history of fever and malaise. Physical findings at that time included several small vesicular lesions on the left side of the soft palate, enlarged left cervical lymph nodes, and hepatosplenomegaly. Laboratory studies disclosed a hemoglobin of 11.8 g/dl, a WBC of 2400/cu mm with 83% polymorphonuclear leukocytes and 7% lymphocytes, and a platelet count of 35,000/cu mm. The bone marrow was normal with all cell lines represented and no evidence of leukemia. Results of a cerebrospinal fluid examination were normal. A "Monospot" test was positive.

Over the next week, he developed high fever, cough, pharyngitis with a whitish tonsillar exudate, and progressive hepatosplenomegaly. On November 9, 1979, the blood showed a hemoglobin of 9.1 g/dl, a WBC of 1300/cu mm with 42% polymorphonuclear leukocytes, 32% lymphocytes, and 16% monocytes, and a platelet count of 17,000/cu mm. A chest roentgenogram showed right middle and left lower lobe pneumonia. The child was admitted to the hospital for antibiotic therapy.

On November 12, 1979, his liver was palpable 6 cm below the right costal margin and his spleen 10 cm below the left costal margin. He had ascites with scrotal and ankle edema. A complete blood count at that time showed a hemoglobin of 9.7 g/dl (maintained by daily transfusions), WBC of 1700 with 30% polymorphonuclear leukocytes, 7% bands, and 49% lymphocytes, and a platelet count of 20,000/cu mm. Additional laboratory findings included a serum albumin of 1.6 g/dl, serum bilirubin of 3.1 mg/dl (direct, 1.4 mg/dl), aspartate aminotransferase of 125 IU/liter, blood urea nitrogen of 15.7 mg/dl, and creatinine of 0.7 mg/dl; the coagulation screen

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³ The abbreviations used are: EBV, Epstein-Barr virus; ALL, acute lymphoblastic leukemia; EBNA, Epstein-Barr nuclear antigen; XLP, X-linked lymphoproliferative syndrome.

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disclosed a prothrombin time of 19.4 sec with a control value of 10.1 sec, a partial thromboplastin time of 50 sec with a control value of 24.4 sec, a thrombin time of 30.6 sec with a control value of 20.5 sec, and a fibrinogen value of 40 units. Fibrin degradation products were not significantly elevated. His bone marrow was hypercellular with increased numbers of mature histiocytes, some demonstrating erythrophagocytosis. He was treated with spironolactone (Aldactone; G. D. Searle and Co., Chicago, Ill.), i.v. albumin, and prednisone, 40 mg/sq m/day for 1 week.

On November 21, 1979, while receiving prednisone, he developed recurrent fever (38°) and had an increase in spleen size to 15 cm below the right costal margin. Prednisone therapy was gradually decreased and finally stopped altogether. His clinical condition continued to deteriorate with high fevers daily, massive splenomegaly 15 cm below the right costal margin, and bilateral cervical lymphadenopathy with nodes measuring 2 x 4 cm. Laboratory studies at that time disclosed a hemoglobin of 7 g/dl despite daily transfusions, a WBC of 900 (30% polymorphonuclear leukocytes, 60% lymphocytes, and 10% monocytes), and a platelet count of 8000/cu mm. His bone marrow contained normal hematopoietic precursors, as well as transformed lymphocytes and numerous histiocytes demonstrating erythrophagocytosis. The uric acid concentration was 13.8 mg/dl, and serum immunoglobulin studies showed an IgG of 2100 mg/dl (normal range, 650 to 1600 mg/dl), an IgA of 330 mg/dl (normal range, 100 to 400 mg/dl), and an IgM of 1500 mg/dl (normal range, 18 to 280 mg/dl), with a polyclonal pattern of IgM by immunoelectrophoresis.

On December 4, 1979, a lymph node biopsy was obtained, and i.v. total parenteral nutrition was begun. From December 4 to 12, the patient continued to have high fever, although his spleen and cervical lymph nodes decreased spontaneously in size.

His pancytopenia progressed during this period, and on December 12, he had 300 WBCs with no polymorphonuclear leukocytes on smears, a hemoglobin of 8.5 mg/dl (transfused), and no detectable platelets on smears. A bone marrow aspirate was extremely hypocellular. From December 12 to 19, his high fever persisted; he developed progressive cardiomegaly, pulmonary edema, and cardiorespiratory failure, which led to his death on December 19.

Results

Pathological Findings. In November 1979, a bone marrow biopsy disclosed that the patient's marrow was hypercellular with panhyperplasia and a shift towards immaturity in the myeloid and erythroid series. Most importantly, a significant histiocytic infiltration was recognized. The cytologically bland histiocytes were phagocytic for erythrocytes, platelets, and rarely leukocytes. In light of the clinical and viral serological features, the histopathological findings were consistent with a virally induced hemophagocytic syndrome (19). No fungi were noted in the sections, smears, or cultures.

When massive lymphadenopathy developed during the next few weeks, a cervical lymph node was excised. The nodal architecture was diffusely effaced by a proliferation of lymphoid cells showing a wide spectrum of maturation, ranging from atypical small lymphocytes to immunoblasts. The histological features seen in this section were not those of any of the well-

characterized malignant lymphomas but were those that have been observed in other cases of EBV infections. The malignant potential of this atypical immunoblastic proliferation is uncertain.

In the ensuing 7 to 10 days, the patient's condition changed from a state of marked lymphoreticular cell proliferation to one of profound lymphoreticular and hematopoietic cell depletion. A bone marrow biopsy performed 8 days after the lymph node biopsy showed marked hypoplasia. It is significant that this change from a hyperproliferative to a hypoproliferative state occurred in the absence of any therapy.

Pertinent postmortem findings were extensive disseminated candidiasis (*C. albicans*), marked pulmonary hemorrhage, profound lymphoid cell and hematopoietic cell depletion, cholecystitis with diffuse hepatic necrosis, fibrosis and cholestasis, and marked splenomegaly with congestion and infarction. Death was attributed primarily to respiratory failure secondary to pulmonary congestion, hemorrhage, and infection.

Viral and Serological Studies. The patient's antibody responses to EBV-related antigens, as determined by immunofluorescence (6, 17), are presented in Table 1. The primary infection appears to have occurred between July and October of 1979. Serum samples obtained in June and July (6/26/79, 7/11/79, and 7/23/79) were negative for antibodies against EBV. By the end of October, IgA, IgM, and IgG antibodies were detectable against viral capsid antigen. IgA and IgM antibody titers gradually decreased, while IgG titers did not change until the development of aplastic anemia. A low IgG anti-diffuse antigen response was observed during the acute phase of infection. Antibodies to EBNA present in low titers on 11/14/79 and 12/1/79 were due to blood transfusions.

Imprints prepared from lymph node tissue 2 weeks before death indicated that ≥50% of the cells were positive for EBNA (Fig. 1) (17, 18). However, EBNA was not detected in imprints of lymph node and spleen obtained at autopsy. This observation coincides with the patient's aplastic state, which developed before death and may reflect degeneration of EBNA-positive cells.

Two days before death, the patient's lymphocytes obtained from peripheral blood responded to phytohemagglutinin stimulation (50% of normal), although the absolute number of lymphocytes was very low (800/cu mm). Only 34% of these cells were erythrocyte rosette positive (normal percentage, 62.1 ± 7.1).

Table 1

Antibody response during EBV infection

Antibody response is presented as reciprocal of the highest dilution showing specific immunofluorescence.

Date	VCA ^a			D IgG	R IgG	EBNA IgG	PB het- erophil
	IgA	IgM	IgG				
6/26/79	ND	ND	<10	<10	<10	<2	Neg
7/11/79	ND	ND	<10	<10	<10	<2	Neg
7/28/79	<10	<10	<10	<10	<10	<2	20
10/31/79	10	160	≥320	<10	<10	<2	1280
11/9/79	40	40	320	10	?	<2	1280
11/14/79	20	10	320	10	?	5	640
12/1/79	<10	<10	160	<10	<10	2	Neg
12/7/79	<10	ND	80-160	<10	<10	<2	ND
12/17/79	<10	ND	80	<10	<10	10	ND
12/19/79	Patient died						

^a VCA, viral capsid antigen; D, diffuse antigen; R, restricted antigen; PB, Paul-Bunnell; ND, not done; Neg, negative.

Discussion

The patient described in this report developed a fatal illness resulting from a primary Epstein-Barr virus infection. Features of the lymph node biopsy specimen obtained at the height of the proliferative phase of the illness did not resemble any well-characterized lymphomas but had histological features observed in certain cases of EBV infection. Greater than 50% of the lymph node cells were positive for EBNA. These findings strongly implicate EBV infection as the causative agent in this patient's final disease.

Fatal lymphoproliferative syndromes have been reported to develop after primary EBV infection, but only rarely, for example in an X-linked recessive pattern (2, 13, 14). Others have occurred in males and females with no family history of fatal EBV infection (3, 4, 20, 24). Our patient, although a male, lacked a documented family history suggestive of XLP; however, 2 maternally related uncles with congenital heart disease had died of pneumonia. He demonstrated anti-viral capsid antigen and anti-early antigen responses and was also similar to patients with XLP in that a low titer of anti-EBNA was noted. The similarities to patients with XLP can be seen elsewhere in this issue in articles by Purtilo *et al.* (15, 16) and Masucci *et al.* (9, 10).

This case is unique in at least 2 important ways. First, the patient demonstrated lymphoproliferation and aplastic anemia phenotypes described previously with fatal EBV infection in XLP (14). He progressed rapidly from a lymphoproliferative disorder to an aroliferative phenotype, with regard to both lymphoreticular and hemopoietic elements. Although he had pancytopenia throughout his illness, his low circulating blood cell counts first appeared to be due to entrapment in an enlarged spleen, since the bone marrow was hypercellular with all cell lines represented. After the lymph node biopsy on December 4, his pancytopenia became much worse. This was at a time when the spleen size was spontaneously decreasing and the bone marrow was hypocellular. These findings suggest that the cause of his pancytopenia had shifted from splenomegaly to decreased production of circulating cells due to marrow hypoplasia.

Second, the prevalence of EBNA in the patient's lymph node cells underwent a striking change. At a time when the lymphoproliferative aspect of the disease was at its height, 2 independent laboratories demonstrated that $\geq 50\%$ of the lymph node cells were EBNA positive. As the clinical phenotype changed, all evidence of EBNA-positive cells was lost. Although we do not fully understand the basis for the patient's response to EBV, it could reflect a disorder in immunoregulation of his specific immune response.

Conceivably, the patient had a subtle immunodeficiency, either secondary to chemotherapy or perhaps genetically based, before EBV infection. Such deficits have been described in several patients with fatal EBV infections (5, 20) and in at least one patient receiving immunosuppressive therapy with subsequent fatal EBV infection (8). After exposure to the virus, the patient might have been unable to maintain a normal cellular immune response to EBV and hence developed an exaggerated proliferation of B-lymphocytes. Moreover, there may have been a deficit in any of 3 previously described cellular immune responses to EBV: lympholysis (14); induction of EBV-specific T-killer cells that destroy infected cells (22); or

excessive production of T-suppressor cell activity that suppresses lymphocyte (23), as well as bone marrow proliferation (1).

The reversal of this clinical picture to a hypoproliferative state coincided with the disappearance of EBNA-positive cells, suggesting a causal relationship between the 2 events. Perhaps the immune mechanisms involved in the control of the EBV infection, while sluggish in starting and accounting for the massive proliferation of EBV-infected cells, later became quite active. Thus, lympholysis and EBV-specific T-cell activity could have led to nearly complete destruction of EBV-infected cells. The bone marrow stem cells may have been affected by the exaggerated immunoregulatory response of suppressor T-cells. It is likely that a combination of these abnormalities, and perhaps others, resulted in the clinical picture observed in our patient.

Acknowledgments

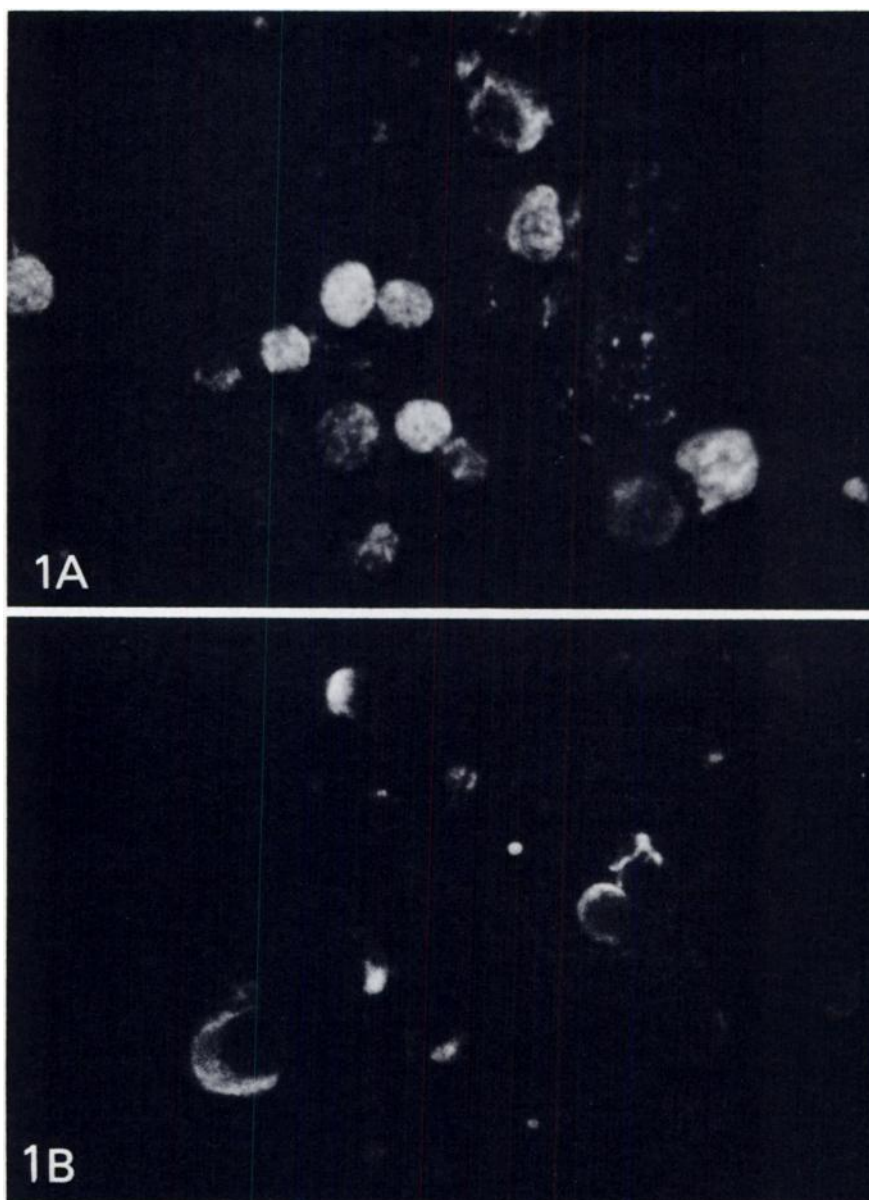
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Fig. 1. Touch preparations from the lymph node biopsy tested by anticomplement immunofluorescence. A, serum with antibodies to EBNA; B, serum without antibodies to EBNA. $\times 1440$.



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