Fatal liver failure: molecular evidence for chronic active Epstein-Barr virus infection

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Abstract

Epstein-Barr virus (EBV) infection is a benign disease, which may occasionally be fatal, particularly in children and immunocompromised patients. Epstein-Barr virus infection is rare in elderly subjects and seems to have a self-limited course. A case of fatal liver failure due to chronic active EBV infection in a 75-year-old man is described. The etiology was established postmortem by cellular expression of EBV-DNA in liver and lymphatic tissue. This patient meets the diagnostic criteria of a case definition for a highly probable "severe chronic active EBV syndrome." Either suspected or unsuspected, the long-term prognosis of this syndrome is poor and its mortality high.

Keywords: Epstein-Barr virus; Infectious mononucleosis; Liver failure

1. Introduction

Epstein-Barr virus [1] (EBV) was discovered 40 years ago in tumor cells from Burkitt lymphoma. Extensive virological and epidemiological studies have shown that EBV causes infectious mononucleosis (IM) and contributes to the pathogenesis of multiple malignancies, including Burkitt lymphoma, Hodgkin lymphoma, primary central nervous lymphoma in patients with AIDS, and nasopharyngeal carcinoma [2]. Atypical courses of the primary infection [3] may vary from clinically benign illnesses, such as chronic fatigue, to aggressive syndromes with features of renal or hepatic failure as well as fulminant T-cell lymphoproliferative disorders [4]. In individuals with a normal immune system, sustained T-cell infection by EBV occurs only rarely, raising the possibility that the infection of T lymphocytes and their subsequent unregulated growth are due, at least in part, to a defect in immune surveillance.

2. Case report

2.1. Clinical history and histologic findings

A 75-year-old man presented with toxic syndrome, pyrexia, bicytopenia, and cholestasis. The patient was receiving treatment of type 2 diabetes, hypertension, and dyslipidemia. He was previously in good condition, with his underlying diseases well controlled, and without any sign of opportunistic infections or other indications of any congenital immunodeficiency; nor had he received immunosuppressive medications. There was no history of exposure to tuberculosis or HIV; however, the patient reported to have had 2 months before a self-limited process with acute onset of fever and general malaise suggestive of a viral upper respiratory illness. Within a period of 2 months, the patient developed toxic syndrome, daily fever, and finally mild hepatosplenomegaly and jaundice, without any demonstrable lymphadenopathy. Laboratory tests showed progressive pancytopenia (white blood cell count, 1.65 × 10^9/L [810 total lymphocytes]; hemoglobin, 86 g/L; platelets, 90 × 10^9/L) and abnormal liver function test (total bilirubin, 2 mg/dL; aspartate transaminase, 89 U/L; alanine transaminase, 96 U/L; γ-glutamyl-transpeptidase, 172 U/L; alkaline phosphatase, 1865 U/L). Blood cultures for both conven-
tional-bacterial and mycobacterial infections obtained at the height of the fever were negative. Epstein-Barr virus antiviral capsid antigen (anti-VCA) immunoglobulin (Ig) G titers were >750 U/mL (upper limit, ≥20); anti-VCA IgM antibodies, 0.13 (upper limit, 0.9); and heterophile, negative (Table 1) [5]. Immunoglobulin M antibodies to cytomegalovirus; antibodies to HIV 1 and 2 and hepatitis A, B, and C viruses; and the serologies for bacterial agents causing prolonged/recurrent fever as Brucella melitensis, Borrelia burgdorferi, Treponema pallidum, Rickettsia conorii, and Coxiella burnetii were all negative. The bone marrow aspirate showed reactive findings, with only 1% of nonclonal B cells (CD19+). Imaging diagnosis including cardiac ultrasonography and the combination of computer tomography and bone 67-gallium scans failed to show any remarkable findings, neither trace of an identifiable acute infection nor tumor. Ultrasound-guided liver biopsy showed mild chronic portal inflammation with involvement of bile duct epithelium and focal necrosis of the lobules (Fig. 1). Those findings were corroborated in the liver postmortem studies where the necrosis was submassive. Well-constituted granulomas in the studied sample were not found; but in this clinical context, the presence of focal lobule necrosis suggested an infectious etiology as first possibility.

Despite the fact that there were a normal chest x-ray and a negative nonboosted tuberculin test, the rapid worsening of the clinical condition and the liver function tests, together with the preliminary report of the hepatic biopsy, led to the suspicion of disseminated tuberculosis. The patient received antitubercular drugs for 2 days, but ultimately died in the intensive care unit from progressive liver dysfunction and further associated multiorgan failure. The macroscopic postmortem examination did not show remarkable alterations in any organ. The microscopic examination of the spleen showed splenic congestion with erythrophagocytosis that was also seen in bone marrow, and hypoplasia of the white pulp. Lymph nodes showed lymphoid depletion with an increased number of plasma cells, and focus of necrosis and fibrosis (Fig. 2). The conventional-bacterial, mycobac-

### Table 1

| EBV disease stage | Production of: |  |
|---|---|---|---|---|---|
| | Anti-VC IgG | Anti-VCA IgM | Anti-NA-1 IgG | Anti-EA-D IgG | Heterophile antibody |
| Susceptible Primary acute | Neg | Neg | Neg | Neg | Neg |
| | Pos/Neg | Pos | Neg | Neg | Pos/Neg |
| Pos | Neg | Neg | Pos | Pos | Pos |
| Prior or remote recovery | Pos | Pos/Neg | Pos | Pos/Neg | Pos/Neg |
| Case report | Pos | Pos | ND | ND | Neg |

Fig. 1. Hematoxylin and eosin staining at ultrasound-guided biopsy performed 3 weeks before patient’s death. Low-power view showing focal necrosis of the lobules.

Fig. 2. Hematoxylin and eosin–stained necropsy sample. Lymph nodes showing lymphoid depletion with an increased number of plasma cells, and focus of necrosis and fibrosis. Mild-power view.

Fig. 3. In situ hybridization for EBV showing massive EBV-DNA expression in liver tissue. Similar features where found in lymph nodes and spleen.
terial, and fungal cultures obtained from all organ samples were negative.

2.2. Molecular studies

The DNA extracted from the necropsy sample was amplified through polymerase chain reaction using the oligonucleotides corresponding to the γ chain of the T-cell receptor, and the sequence obtained by electrophoresis did not show any image compatible with a clone pattern for the T-cell receptor. The real-time polymerase chain reaction using the sequence IS6110 of Mycobacterium tuberculosis did not show any plot of amplification for M tuberculosis either. Conversely, in situ hybridization for EBV performed according to standard protocols proved massive EBV-DNA infection in lymph nodes, spleen, and liver (Fig. 3).

3. Discussion

Hepatic manifestations of EBV infection consist largely of self-limited elevations of hepatocellular enzyme levels, which are present in 80% to 90% of the cases of IM [6]. Epstein-Barr virus entry into B lymphocytes is believed to be critically dependent on the expression of a specific EBV receptor, the CD21 molecule, a 145-kd glycoprotein also termed the C3d receptor or CR2 [7]. However, in recent years, the range of host cells known to be susceptible to EBV infection has steadily expanded to include epithelial cells, T lymphocytes, and hepatocytes. The EBV receptor has not yet been documented on hepatocytes. The mechanism of the infection of the hepatocytes by EBV remains unknown. Acute hepatic failure caused by primary EBV infection is a rare event poorly documented in adults [8]; hepatic involvement of chronic active EBV infection should be considered as differential diagnosis in cases showing liver dysfunction with clinical and biochemical features of an underlying infectious disease and with negative cultures and/or inconclusive serologies. Although death from IM is rare, a fatal course of IM may occur either as a result of the overwhelming EBV infection or from complications of diseases such as lymphoproliferative disorders, meningoencephalitis, splenic rupture, upper airway obstruction, myocarditis, or hepatic failure [9]. A persistent EBV infection with demonstrable virus in lymph nodes, spleen, thymus, and other organs may occur in apparently healthy persons; but primary and secondary or acquired immunodeficiency syndromes have also been described. Serologic tests, as in this case, based on enzyme-linked immunosorbent assay and immunofluorescence assay are commonly used to distinguish acute vs nonacute EBV disease; but its diagnosis by serologic means is—when this condition is not strongly suspected—extremely tenuous. A heterophile test is negative and atypical lymphocytes are absent in this syndrome. Although serologic methods are well characterized and reliable, they require an aliquot of sample and reagents for each antigen tested, thus necessitating a panel of assays to complete the evaluation [10,11]. Because the patient sample has to be divided into aliquots multiple times, this can lead to what is called in the laboratory field pour off errors. Because chronic EBV infection was not suspected in this case and the patient died within 4 weeks from his admission, not allowing the possibility of repeating the tests, the serology did not help us to reach the diagnosis.

In 1948, Isaacs described a prolonged clinical course of IM lasting from months to years [12]. During the late 1970s and the early to middle 1980s, several groups reported cases of a protracted illness usually preceded by IM but with persistent fatigue, headaches, myalgia, lymphadenopathy, and intermittent or low-grade fever. In 1978 and in 1984, Virelizier et al [13] and Joncas et al [14] described, respectively, similar life-threatening cases of a severe type of chronic active EBV infection. The affected patients were young adults who had a chronic disease characterized by fever, lymphadenopathy, splenomegaly, and extremely high EBV antibody titers against VCA and early antigen. Despite the fact that there were rare patients with this syndrome who had developed severe symptoms overtly associated with an active EBV infection, Okano et al [15] proposed in 1991 that this disorder be designated “severe chronic active EBV syndrome” (SCAEVB). Patients with SCAEBV, usually children and young adults, can often develop life-threatening complications over the course of months or several years (Table 2).

We described a case of fatal liver dysfunction after chronic EBV infection in a previously healthy adult. The case progressed toward viral sepsis, multiple organ failure, and death. Central to the syndrome was the laboratory findings of hepatic involvement and hemophagocytosis. According to Okano et al, this patient fulfills the diagnostic criteria of a case definition for SCAEBV. The serology was not helpful in this case, but the pathologic findings were. The data published up to now indicate that the variety of clinical manifestations induced by EBV at least partly depend on the immune response elicited in the host and not on virus replication per se. Now we know that in situ hybridization techniques may be critically helpful to reach the etiology of several infective conditions such as EBV and that they also may reveal viral mechanistic insights to gain a better understanding of the biology of this infection in vivo.

<table>
<thead>
<tr>
<th>Category</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Clinical</td>
<td>Intermittent fever, lymphadenopathy, and hepatosplenomegaly.</td>
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<tr>
<td>Hematologic</td>
<td>Anemia, thrombocytopenia, lymphopenemia or lymphocytopenia or lymphophagocytosis, neutropenia, and polyclonal gammopathy.</td>
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<tr>
<td>Virological</td>
<td>Elevated antibody titers and positivity for antibodies to EBV-related antigens (VCA IgG, ≥ 5120; VCA IgA, positive; EA [D] IgG, ≥ 640; EA [D] IgA, positive; and EA [D] and EA [R] IgG, ≥ 640) and/or detection of EBV genomes in affected tissues.</td>
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<tr>
<td>Other</td>
<td>Chronic illness that cannot be explained by other known disease processes.</td>
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Table 2

Diagnostic criteria of a case definition for SCAEBV [15].
References