The Molecular Characterization of Fatal Infectious Mononucleosis

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Key Words: Fatal infectious mononucleosis; Epstein-Barr virus; Gene rearrangements; Southern blot analysis; Polymerase chain reaction

Abstract

We describe a retrospective study of 4 cases of sporadic fatal infectious mononucleosis (IM), 1 case of fatal IM, and 1 case of sporadic severe IM. Patients were 26 months to 17 years old; 3 were male. Five died of complications of IM. All 5 of these patients had the Epstein-Barr virus (EBV) present in examined tissue specimens; EBV was monoclonal in 3 patients and biclonal in 1. EBV clonality studies were not performed in the remaining patient. All 5 patients also had monoclonal gene rearrangements. The sixth patient survived despite a life-threatening clinical course; EBV was oligoclonal, and gene rearrangements were not detected.

EBV clonality and gene rearrangement studies may be useful for predicting which patients with clinically aggressive IM are at highest risk for fatal outcome. Patients in whom IM has a fatal outcome are more likely to have monoclonal or biclonal EBV and immunoglobulin heavy chain or T-cell receptor gene rearrangements. In contrast, patients with nonfatal IM may lack monoclonal EBV and monoclonal rearrangements of the aforementioned genes. The reasons EBV induces a monoclonal proliferation only in some patients remain to be elucidated.

Epstein-Barr virus (EBV) is a ubiquitous DNA virus that infects most of the world’s population. It is implicated in a multitude of human diseases from frankly malignant, monoclonal disorders, such as Burkitt lymphoma, to borderline malignant, occasionally polyclonal disorders, such as post-transplant lymphoproliferative disorders. Fortunately, the most common pattern of EBV infection is a clinically silent, childhood infection. When primary infection occurs during adolescence or adulthood, EBV may cause infectious mononucleosis (IM), a typically benign, self-limited disorder. Clinically, IM is characterized by fever, pharyngitis, lymphadenopathy, and, often, splenomegaly; morphologically, it is characterized by exuberant, polyclonal lymphoproliferation. In this disorder, EBV infects B lymphocytes, inducing a cytotoxic T-cell response that eventually curbs viral proliferation and restrains the immune response [Figure 1].

In a small number of patients, however, IM pursues a much more aggressive course: early lymphocytosis in peripheral blood is soon followed by lymphocytic invasion of vital organs, hemophagocytosis, massive marrow necrosis, and severe pancytopenia.\(^\text{1-5}\) Such patients usually die of opportunistic infections and/or hemorrhage within several weeks.\(^\text{1-5}\) The majority of patients with severe or fatal IM (FIM) are immunocompromised, either by an inherited immunodeficiency disorder, such as X-linked lymphoproliferative syndrome, or from administration of immunosuppressive drugs.\(^\text{6-9}\) Thus, they are unable to mount the immunologic response necessary to contain EBV proliferation (Figure 1). A small number of patients with severe or fatal IM, however, apparently are immunocompetent before EBV infection. Cases of sporadic fatal IM (SFIM) occurring in these patients number approximately
1 in 3,000 cases of IM; in these cases, the factors leading to uncontrolled lymphoproliferation are more difficult to define.3,4

We studied 5 previously healthy patients and 1 immunodeficient patient who developed severe or fatal IM. The polymerase chain reaction (PCR) and Southern blot analysis were used to evaluate EBV clonality and immunoglobulin heavy chain (IgH) gene and T-cell receptor (TCR) gene rearrangement status. We also reviewed the morphologic findings in available tissues in each case. Molecular and morphologic findings were correlated with each patient’s clinical course, as obtained from medical records.

Materials and Methods

EBV Detection

Two methods were used to detect and characterize EBV within the patient samples: polymerase chain reaction (PCR) and Southern blot analysis.

Polymerase Chain Reaction

DNA was isolated from the specimen through the use of the PureGene System (Gentra Systems, Minneapolis, MN). PCR was performed in a total volume of 50 µL with 2 µL of sample containing 0.15 ng of DNA. Other components of the PCR cocktail included the following: a 0.25-mmol/L concentration of magnesium chloride, a 0.025-mmol/L concentration of each deoxynucleoside triphosphate, and 1 U of AmpliTaq (Applied Biosystems, Foster City, CA). To amplify EBV, a 0.15-mmol/L concentration of an EBV sense primer (5’-GCAGTAAAGTGCTCCTCGG-3’) and a 0.15-mmol/L concentration of an EBV antisense primer (5’-CCAGAAATAGCTGAGGACC-3’), which amplify a 401-base-pair portion of the BamH1 W fragment of EBV, were used. As an internal control, a 0.3-mmol/L concentration of a p53 sense primer (5’-TATCCCTAGTAATGTTAATC-3’) and a 0.3-mmol/L concentration of a p53 antisense primer (5’-AAGTGAATCTGAGGCATAAC-3’), which amplify exon 8 of the p53 gene, were included in each reaction. Each PCR run included an FJO cell line sample as a positive control.

DNA was amplified using the following thermal cycler parameters: 94°C for 3 minutes; 30 cycles of 94°C for 1 minute, 59°C for 30 seconds, and 72°C for 30 seconds; 72°C for 5 minutes; and a 4°C soak. PCR products were electrophoresed on a Novex precast 6% polyacrylamide gel (Novex, San Diego, CA) at 100 V for 1 hour. Products were analyzed after staining the gels in ethidium bromide.

Southern Blot Analysis

Southern blot analysis was performed using a 7.7-kb fused terminal fragment probe.10 Briefly, 10 µg of sample DNA was digested with the BamH1 restriction endonuclease. The fragments were electrophoresed through a 0.6% agarose gel and transferred to a Zetabind membrane (Cuno, Meriden, CT). The probe was labeled with phosphorus 32 by nick translation.11 The membrane was hybridized with the probe overnight at 42°C. The membrane was then washed in 2x standard saline citrate and 0.1% sodium dodecyl sulfate for 15 minutes at room temperature, followed by two 30-stringency washes, each with 0.1% standard saline citrate and 0.1% sodium dodecyl sulfate at 60°C.

Gene Rearrangement Detection

Gene rearrangement detection was performed using PCR, followed by Southern BLOT analysis when necessary for confirmation, using methods outlined by Coad et al.12,13

Results

Clinical Findings

Four patients with SFIM, 1 patient with FIM, and 1 patient with sporadic severe IM (SSIM) were included in the study. Patients ranged in age from 26 months to 17 years (mean, 10 years, 10 months). Five of 6 patients were previously healthy, with no known immunodeficiencies or receipt of immunosuppressive therapy. One patient had a history of concentration of each deoxynucleoside triphosphate, and 1 U of AmpliTaq (Applied Biosystems, Foster City, CA). To amplify EBV, a 0.15-mmol/L concentration of an EBV sense primer (5’-GCAGTAAAGTGCTCCTCGG-3’) and a 0.15-mmol/L concentration of an EBV antisense primer (5’-CCAGAAATAGCTGAGGACC-3’), which amplify a 401-base-pair portion of the BamH1 W fragment of EBV, were used. As an internal control, a 0.3-mmol/L concentration of a p53 sense primer (5’-TATCCCTAGTAATGTTAATC-3’) and a 0.3-mmol/L concentration of a p53 antisense primer (5’-AAGTGAATCTGAGGCATAAC-3’), which amplify exon 8 of the p53 gene, were included in each reaction. Each PCR run included an FJO cell line sample as a positive control.

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multiple, severe, recurrent infections and serologic evidence of immune deficiency (decreased IgG subclasses II and IV). The 4 patients with SFIM died of complications related to the EBV infection or its treatment, including hemophagocytic syndrome (3 patients) and multiorgan system failure (1 patient). The patient with FIM in the setting of immune deficiency died of respiratory distress syndrome.

### Table 1
Clinical, Morphologic, and Molecular Data

<table>
<thead>
<tr>
<th>Case No./Sex/Age</th>
<th>Diagnosis</th>
<th>Bone Marrow</th>
<th>Lymph Node</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/12 y</td>
<td>SFIM</td>
<td>Polymorphous lymphoid infiltrates; necrosis</td>
<td>Polymorphous lymphoid infiltrates</td>
<td>Polymorphous lymphoid infiltrates; necrosis</td>
<td>Hemophagocytosis; lymphocytes depletion; histiocytosis</td>
<td></td>
</tr>
<tr>
<td>2/F/3 y, 9 mo</td>
<td>SFIM</td>
<td>Hemophagocytosis; lymphocytes depletion</td>
<td>—</td>
<td>Polymorphous lymphoid infiltrates</td>
<td>Hemophagocytosis; lymphocytes depletion; histiocytosis</td>
<td></td>
</tr>
<tr>
<td>3/F/17 y</td>
<td>SFIM</td>
<td>Hemophagocytosis; lymphocytes depletion; histiocytosis</td>
<td>Hemophagocytosis; histiocytosis</td>
<td>Histiocytosis; necrosis</td>
<td>Histiocytosis; lymphocytes depletion</td>
<td></td>
</tr>
<tr>
<td>4/M/9 y</td>
<td>SFIM</td>
<td>—</td>
<td>—</td>
<td>Polymorphous lymphoid infiltrates; histiocytosis</td>
<td>Histiocytosis; necrosis</td>
<td>—</td>
</tr>
<tr>
<td>5/F/17 y</td>
<td>SSIM</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6/M/26 mo</td>
<td>FIM</td>
<td>—</td>
<td>Polymorphous lymphoid infiltrates; necrosis</td>
<td>Polymorphous lymphoid infiltrates</td>
<td>Polymorphous lymphoid infiltrates; necrosis</td>
<td>Polymorphous lymphoid infiltrates</td>
</tr>
</tbody>
</table>

FIM, fatal infectious mononucleosis; IgH, immunoglobulin heavy chain; ND, not done; SFIM, sporadic FIM; SSIM, sporadic severe infectious mononucleosis; TCR, T-cell receptor.

### Morphologic Features

Histologic examination was performed in all 6 cases. In 4 of 6 cases, autopsies were performed; in the remaining 2 cases, limited organ biopsies were performed. Three of 4 patients with SFIM (cases 1-3) had remarkably similar histopathologic findings, consisting of the following: (1) polymorphous lymphoid infiltrates, (2) histiocytosis, (3) necrosis, (4) lymphocyte depletion, and/or (5) hemophagocytosis. In all 3 patients, these features were widespread. The lymph nodes, spleen, liver, and bone marrow were consistently involved, showing 2 or more of the aforementioned features Image 1 through Image 4.

Other organs, such as lung, kidneys, and pancreas, showed variable involvement. In the remaining patient with SFIM (case 4), an autopsy was not performed. Liver and lung biopsies for this patient revealed histiocytosis, lymphoid infiltrates, and/or necrosis.

In the patient with SSIM (case 5), morphologic examination of the tonsils revealed a diffuse polymorphous lymphoid infiltrate. However, no histiocytosis, necrosis, lymphocyte depletion, or hemophagocytosis were present.

Finally, in the patient with FIM (case 6), examination of autopsy material revealed widespread, atypical, polymorphous lymphoid infiltrates in virtually all organs examined. Although necrosis was present in a few organs, histiocytosis, lymphocyte depletion, and hemophagocytosis were absent.
In each patient, the morphologic abnormalities seemed to be commensurate with the degree of clinical severity. In the 4 cases of SFIM described herein, the morphologic features were similar to those described by others. Notably, most involved organs contained polymorphous lymphoid infiltrates, necrosis, hemophagocytosis, and histiocytosis; occasionally, lymphocyte depletion was present. However, not all features were present in each organ in each case. The morphologic features in the case of FIM occurring in an immunodeficient patient were slightly different; widespread polymorphous, strikingly atypical lymphoid infiltrates, occasionally associated with necrosis, were prominent. In contrast, the patient with SSIM had only polymorphous lymphoid infiltrates, with none of the aforementioned features.

### Table: Gene Rearrangements

<table>
<thead>
<tr>
<th>Other Organ</th>
<th>Epstein-Barr Virus</th>
<th>B-Cell</th>
<th>T-Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood: reactive lymphocytosis</td>
<td>Monoclonal</td>
<td>Negative</td>
<td>TCR beta TCR gamma</td>
</tr>
<tr>
<td>Adrenals: polymorphous lymphoid infiltrates</td>
<td>Monoclonal</td>
<td>Negative</td>
<td>TCR beta</td>
</tr>
<tr>
<td>Thyroid: histiocytosis</td>
<td>Monoclonal</td>
<td>IgH</td>
<td>Negative</td>
</tr>
<tr>
<td>—</td>
<td>Biclonal</td>
<td>Negative</td>
<td>TCR beta</td>
</tr>
<tr>
<td>Tonsils: polymorphous lymphoid infiltrates</td>
<td>Oligoclonal</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Gastrointestinal, kidneys, pancreas: polymorphous lymphoid infiltrates</td>
<td>Present; clonality ND</td>
<td>IgH</td>
<td>Negative</td>
</tr>
</tbody>
</table>

[Image 2i (Case 1) Liver. Polymorphous lymphoid infiltrate composed of small lymphocytes and large, atypical lymphocytes (H&E, ×40).]

[Image 3i (Case 1) Liver. Polymorphous lymphoid infiltrate composed of small lymphocytes and large, atypical lymphocytes. Note hemophagocytosis (H&E, ×60).]

[Image 4i (Case 1) Spleen. Lymphocyte depletion in white pulp with scattered atypical lymphocytes (H&E, ×40).]
Molecular Studies

Epstein-Barr Virus

PCR for EBV was performed in all 6 cases; EBV was present in each Image 5. Furthermore, in the 5 patients with fatal disease, Southern blot analysis revealed monoclonal EBV in 3 and bclonal EBV in 1 Image 6. In the remaining patient, DNA was not present in sufficient quantity to perform Southern blot analysis. Southern blot analysis revealed oligoclonal EBV in the patient with SSIM.

Gene Rearrangements

PCR and Southern blot analysis were performed on tissue specimens from all 6 patients (Table 1). All 5 patients with fatal disease had monoclonal gene rearrangements; 2 patients had rearrangements of the TCR beta chain, 1 had rearrangement of the TCR beta and TCR gamma chains, and 2 had rearrangements of the IgH gene Image 7. Clonal gene rearrangements were not detected in the patient with SSIM.

Discussion

FIM is associated most frequently with EBV infection of an immunocompromised host. The 3 most common causes of immunocompromise are congenital disorders such as X-linked lymphoproliferative disorder, immunosuppressive therapy, and AIDS. There also is increasing literature describing fatal EBV infection of previously healthy individuals. In both vulnerable and previously healthy patients, the clinical and pathologic features of FIM are similar, with the majority of patients dying of organ invasion by proliferating lymphocytes and/or viral associated hemophagocytic syndrome.

Before a report published in 1988, in which EBV-containing T-cell lymphomas were described, it was generally accepted that EBV demonstrated B-cell lymphotropism. Indeed, EBV infection of B cells has been documented in processes ranging from benign (eg, lifelong persistence of EBV-transformed B cells in an asymptomatic, previously infected host) to frankly malignant (eg, EBV-associated Burkitt lymphoma). Since the
initial report, EBV infection of T cells has been well
documented. More recently, EBV infection of T cells
has been documented in cases of life-threatening
and fatal fatal IM. Molecular studies in cases of SFIM have
demonstrated monoclonal IgH rearrangements
and/or monoclonality detected by Southern blot analysis of the
EBV genome. However, there is an increasing literature
describing oligoclonal and monoclonal T-cell
terminations in cases of acute and/or life-threatening
IM. The association between life-threatening or fatal IM
and abnormal T-cell proliferation has led to the suggestion
that atypical T-cell proliferation is responsible for
the aggressive nature of SFIM, In many of these
studies, however, thorough molecular analyses including
TCR, immunoglobulin, and EBV clonality analyses have
not been performed. In the series of patients described
herein, each of the 4 patients with SFIM demonstrated
monoclonality or biclonality as detected with the EBV
probe; 3 of these patients also had monoclonal TCR gene
rearrangements, while the remaining patient demonstrated
a monoclonal IgH rearrangement. The patient
with SSIM demonstrated oligoclonality with the EBV
probe, and no T-cell or IgH rearrangements were
detected. Results from this series of patients suggest that
poor outcomes in cases of apparently life-threatening IM
are most accurately predicted on a molecular basis by
EBV clonality studies and that TCR or IgH gene
rearrangement studies alone are inadequate. Currently in
our laboratory, we use a 2-step approach for the molec-
ular characterization of SSIM and SFIM. The initial step
includes PCR analysis for the detection of EBV
sequences and TCR and IgH gene rearrangements; for the
cases in which EBV is detected, a second step involving
EBV clonality studies by Southern blot analysis is
performed. We believe this to be the most efficient
approach for the molecular evaluation of these cases.

Patients with SFIM often undergo a rapid clinical
course that is unresponsive to treatment. There is
evidence, as well as speculation, in the literature
suggesting that T-cell cytokines are responsible for the
viral-associated hemophagocytic syndrome that results in
the death of these patients. However, neither the precise
molecular mechanisms that are responsible for the
progression of acute IM to SFIM nor early markers of
aggressive disease have been elucidated. It is likely that a
prospective multicenter study involving clinical findings
and outcomes, hematologic studies, cytokine studies, and
molecular analyses will be required to provide insights
into the molecular biology, early definitive diagnosis, and
effective treatment of SFIM. Until that time, molecular
diagnostics will continue to have an important role in the
study of FIM.

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