Epstein-Barr Virus–Associated Hemophagocytic Syndrome: Virological and Immunopathological Studies

By John L. Sullivan, Bruce A. Woda, Henry G. Herrod, Gerald Koh, Fred P. Rivara, and Carel Mulder

The virus-associated hemophagocytic syndrome (VAHS) is a disorder characterized by a benign, generalized histiocytic proliferation, with marked hemophagocytosis associated with systemic viral infections. We have studied the virological and immunopathological events occurring in two children experiencing Epstein-Barr VAHS. Neither of the patients had an underlying immunodeficiency and both recovered from their disease and are completely well one year after follow-up. In each patient, evidence for primary Epstein-Barr virus (EBV) infection was documented with a typical humoral immune response, including IgM antibody directed against virus capsid antigen. EBV was demonstrated in lymphoreticular tissues by electron microscopy and molecular hybridization studies. Permissive EBV infection was suggested by the finding of mature virus particles and linear viral DNA in lymphoreticular tissues. Immunopathological studies demonstrated complete effacement of lymph node architecture by a marked proliferation of immunoblasts in patient 1 and infiltration and effacement of the lymph node architecture with benign-appearing histiocytes in patient 2. Atypical lymphocytes characteristic of acute EBV infection were notably absent in the peripheral blood of both patients and cytotoxic T cells, which normally lyse EBV-infected B cells, were also absent from the peripheral circulation. Our observations suggest that EBV-induced VAHS may be the result of an increased virus burden in the face of immunoregulatory cell imbalances.

Material and Methods

Patients

Two patients were seen, one at the University of Massachusetts Medical Center, Worcester, and one at the LeBonheur Children's Hospital, Memphis. Neither of the patients had a specific immune deficiency, malignancy, or other known predisposition associated with VAHS.

Virological Studies

Antibody titers to EBV capsid antigen, early antigens, and Epstein-Barr nuclear antigen (EBNA) were determined by established methods. In each of these assays, EBV-uninfected cell lines served as controls for nonspecific immunofluorescence. Known positive and negative control sera were tested in parallel. Spontaneous lymphoblastoid cell lines were established as previously described. Touch preparations made from lymphoid tissues were fixed in acetone and methanol and were stained for the presence of EBNA by the method of Reedman and Klein.

Modified Eckhardt Gel Technique

We have recently described a modification of Eckhardt's electrophoretic technique for detecting bacterial plasmids to permit the detection of circular and linear extrachromosomal herpesvirus genomes in mammalian cells. In brief, 0.2 to 2.0 x 10⁶ viable mononuclear cells are suspended in 0.1 mL of a Tris-boric acid, EDTA (TBE) buffer containing 15% Ficoll, 2 Kunitz U/mL RNase (type 1A, Sigma Chemical Company, St. Louis), and 0.01% bromophenol blue. The cell suspensions (0.075 mL) are then loaded into a well of a vertical 0.8% agarose gel prepared in TBE. Lysis buffer (TBE containing 5% Ficoll, 1% SDS, 1 mg/mL of pronase, and 0.05% xylene cyanol green) is then carefully layered over the cell-sample layers.

Electrophoresis is started at 0.8 V/cm for three hours and is then increased to 7.5 V/cm for 14 hours. Gels are stained with ethidium bromide (1 g/mL) and photographed under ultraviolet (UV) light. The DNA in the gel is then transferred to nitrocellulose filters, and the filter is hybridized to ⁵²P-labeled cloned fragments of EBV-DNA and autoradiographed on Kodak XR-2 x-ray film. The BAM HI W (internal repeat) cloned EBV-DNA fragment was used in these studies (pDK-52, kindly supplied by Dr Elliot Kieff, University of Chicago).
Immunological Studies

Cell preparations. Peripheral blood mononuclear cells (PBMCs) were obtained from heparinized venous blood and separated by Ficoll/Hypaque density-gradient centrifugation. Bulk T cell cultures were prepared by placing 0.5 to 1.0 x 10^6 PBMCs in 2 mL of medium containing 30% purified lectin-free T cell growth factor (provided by Dr Kendall A. Smith, Dartmouth Medical School, Hanover, NH). T cell growth was monitored with the use of an inverted microscope, and bulk cultures were analyzed by morphology; staining reactions were performed with monoclonal antibodies.

Lymphocyte surface markers. Standard immunofluorescence techniques were used to study surface-membrane markers. T cell populations were enumerated, using indirect immunofluorescence with the monoclonal antibodies T101 (pan T cell, Hybritech, La Jolla, Calif); OKT1 (pan T cell), OKT3 (T helper/inducer), OKT4 (T cytotoxic/suppressor), OKM1 (monocytes and NK cells) (Ortho Pharmaceutical, Raritan, NJ); and Leu-7 (HNK1, NK cell) (Becton Dickinson, Sunnyvale, Calif). HLA-DR antigens were detected using heterologous antisera directed against a common HLA-DR determinant (anti-p23, kindly provided by Dr Robert Humphreys, University of Massachusetts Medical School, Worcester).

Lymphocyte proliferation and cytotoxicity studies. Lymphocyte proliferation studies were performed in microtiter wells as described using phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM) as stimulants. Assays for EBV-specific killing and natural killer (NK) cells were performed as described.

Case Reports

The clinical criteria for diagnosis including the most salient features of each case are shown in Table 1.

Patient 1. A 30-month-old Portuguese boy was transferred to the University of Massachusetts Medical Center in Worcester with a three-week history of lymphadenopathy and fever. He was in good health until three weeks before admission, when during a visit to Portugal, he developed fever and lymphadenopathy. On returning home, he was taken to his pediatrician and admitted to a local hospital for evaluation. Initial laboratory results included a hemoglobin of 11.7 g/dL, WBC count 5,200/μL, and a platelet count of 17,000/μL. Other serum values included an SGOT of 69 and a total bilirubin of 11.7 g/dL, WBC count 5,200/μL with 27% neutrophils, and a platelet count of 145,000/μL. A chest radiograph showed a right upper lobe infiltrate. The cerebrospinal (CSF) fluid was normal. Bacterial and fungal cultures of blood, urine, CSF, and bone marrow showed no growth. Febrile agglutinins were absent, as were antibodies against histoplasmosis, blastomycosis, aspergillosis, toxoplasmosis, cytomegalovirus, adenovirus, hepatitis A, and hepatitis B. Her serum was negative for Pneumocystis carinii antigen. Her EKG showed signs of mild myocarditis. Bone marrow examination one week after admission showed erythroid hyperplasia and hemophagocytosis. A percutaneous needle biopsy of the liver done on the eighth hospital day showed fatty metamorphosis and portal infiltration by lymphocytes and histiocytes. Electron microscopy revealed herpes-like viral particles in the lymphocytes in the portal area (Fig 1). These particles were aligned along the nuclear membrane and had the appearance of immature EBV particles 80 to 90 nm in length and width. The patient developed progressive hepatosplenomegaly with deterioration of coagulation. Admission laboratory studies revealed a hemoglobin of 9.8 g/dL, a WBC count of 2,700/μL, and a platelet count of 87,000/μL. Cells obtained from the lymph node biopsy were positive for EBNA. Bone marrow aspirate and biopsy showed a hyperplastic marrow with infiltration by histiocytes. After the diagnosis of acute EBV infection was established, the patient was begun on acyclovir 1,500 mg/m²/d in three divided doses for a ten-day course. Over the next three days, he became increasingly lethargic. On the fourth hospital day, his values were: SGOT, 168; SGPT, 206; prothrombin time, 39 seconds with a control of 11 seconds; and partial thromboplastin time was >150 seconds compared with a control of 30 seconds. The patient received vitamin K and fresh-frozen plasma. Over the next three days, the patient’s clinical condition further deteriorated, with a drop of his hematocrit from 34% to 24%; the coagulopathy persisted. The patient’s temperature remained between 39°C and 40°C. On the seventh day of hospitalization, the patient was more alert. Over the next two days, he began to take oral fluids and his WBC count rose to 7,300/μL, with a normal differential. His platelet count slowly rose to 130,000/μL. On the 13th day of hospitalization, and his hemoglobin was stable at 11 g/dL. The patient became afebrile and his hepatosplenomegaly began to resolve. On day 15 of hospitalization, the patient was discharged. Follow-up two weeks after discharge showed that the patient was completely well. There was no evidence of hepatosplenomegaly, and only a few small palpable anterior cervical lymph nodes remained. Examination one year later revealed a healthy 3-year-old boy with no evidence of chronic illness.

Patient 2. A 6-month-old black female was admitted to LeBonheur Children’s Medical Center in Memphis with fever and rhinorrhea, worsening cough, and irritability of two weeks’ duration. At 2 months of age, the child had been diagnosed as having congenital syphils, for which she was treated; she recovered.

On admission to the hospital the infant was irritable, with a temperature of 40.5°C, with rhinorrhea, mild periorbital edema, and bilateral expiratory wheezes. She had small palpable lymph nodes in the cervical, submandibular, axillary, and inguinal regions. Her liver was palpable 1 cm below the right costal margin, and the spleen was palpable 2 cm below the left costal margin. Laboratory data on admission included a hemoglobin of 8.7 g/dL, WBC count of 12,700/μL with 2% bands, 20% neutrophils, 72% lymphocytes, and 6% monocytes. The platelet count was 145,000/μL. A chest radiograph showed a right upper lobe infiltrate. The cerebrospinal (CSF) fluid was normal. Bacterial and fungal cultures of blood, urine, CSF, and bone marrow showed no growth. Febrile agglutinins were absent, as were antibodies against histoplasmosis, blastomycosis, aspergillosis, toxoplasmosis, cytomegalovirus, adenovirus, hepatitis A, and hepatitis B. Her serum was negative for Pneumocystis carinii antigen. Her EKG showed signs of mild myocarditis. Bone marrow examination one week after admission showed erythroid hyperplasia and hemophagocytosis. A percutaneous needle biopsy of the liver done on the eighth hospital day showed fatty metamorphosis and portal infiltration by lymphocytes and histiocytes. Electron microscopy revealed herpes-like viral particles in the lymphocytes in the portal area (Fig 1). These particles were aligned along the nuclear membrane and had the appearance of immature EBV particles 80 to 90 nm in length and width. The patient developed progressive hepatosplenomegaly with deterioration of coagulation. Admission laboratory studies revealed a hemoglobin of 9.8 g/dL, a WBC count of 2,700/μL, and a platelet count of 87,000/μL. Cells obtained from the lymph node biopsy were positive for EBNA. Bone marrow aspirate and biopsy showed a hyperplastic marrow with infiltration by histiocytes. After the diagnosis of acute EBV infection was established, the patient was begun on acyclovir 1,500 mg/m²/d in three divided doses for a ten-day course. Over the next three days, he became increasingly lethargic. On the fourth hospital day, his values were: SGOT, 168; SGPT, 206; prothrombin time, 39 seconds with a control of 11 seconds; and partial thromboplastin time was >150 seconds compared with a control of 30 seconds. The patient received vitamin K and fresh-frozen plasma. Over the next three days, the patient’s clinical condition further deteriorated, with a drop of his hematocrit from 34% to 24%; the coagulopathy persisted. The patient’s temperature remained between 39°C and 40°C. On the seventh day of hospitalization, the patient was more alert. Over the next two days, he began to take oral fluids and his WBC count rose to 7,300/μL, with a normal differential. His platelet count slowly rose to 130,000/μL. On the 13th day of hospitalization, and his hemoglobin was stable at 11 g/dL. The patient became afebrile and his hepatosplenomegaly began to resolve. On day 15 of hospitalization, the patient was discharged. Follow-up two weeks after discharge showed that the patient was completely well. There was no evidence of hepatosplenomegaly, and only a few small palpable anterior cervical lymph nodes remained. Examination one year later revealed a healthy 3-year-old boy with no evidence of chronic illness.

Table 1. Clinical Criteria Suggestive of Virus-Associated Hemophagocytic Syndrome (VAHS)

<table>
<thead>
<tr>
<th>Clinical and Laboratory Findings</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
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<tbody>
<tr>
<td>Fever</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
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<td>+</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>+</td>
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<td>Splenomegaly</td>
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<tr>
<td>Lymphadenopathy</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Abnormal liver function tests</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>+</td>
<td>+</td>
</tr>
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</table>

Adapted from Risdall et al, Cancer 44:993, 1979.
hospital days, the patient underwent exchange transfusions that transiently corrected the clotting abnormalities but did not alter the basic course. After 18 days of hospitalization, a serum specimen from the fifth hospital day was reported to have an IgM titer to EBV capsid antigen of 1:40. A bone marrow examination revealed hypocellularity. Because of the deteriorating condition of the patient and the strong evidence for an EBV infection, the patient was begun on acyclovir on the 18th hospital day. She was placed on 1,500 mg/m²/24 h in three divided doses; when decreased creatinine clearance occurred after 24 hours, the dose was decreased to 1,000 mg/m²/24 h for a total of nine days. The patient gradually improved with clinical stabilization, cessation of fever, and improvement in hematologic parameters. There was no need for further transfusions. The hepatosplenomegaly began to resolve. A repeat liver biopsy done on the 40th hospital day showed smaller portal infiltrates composed predominantly of mature lymphocytes.

The patient was discharged on the 58th hospital day. One month after discharge, her liver and spleen were palpable 2 cm below the costal margin. She was gaining weight normally and developing well. Her clotting studies were normal, but she continued to have mildly abnormal liver function tests, with an SGOT of 116. Follow-up one year later revealed a healthy 18-month-old child with no evidence of chronic illness or immunodeficiency.

**RESULTS**

**Pathological Studies**

**Peripheral blood.** Examination of peripheral blood smears from both patients were remarkable in that neither showed the classical atypical lymphocytosis usually seen during acute EBV-induced infectious mononucleosis. Rare plasmacytoid lymphocytes were identified in the peripheral blood.

**Bone marrow.** In patient 1, the bone marrow biopsy showed a hypercellular marrow resulting from increased hematopoiesis and the focal accumulation of mature histiocytes. The myeloid series was not left-shifted. Megakaryocytes were normal in number. There was a marked increase in benign-appearing mononuclear phagocytes, which sometimes occurred in clusters. In patient 2, the initial bone marrow examination performed on the seventh day of hospitalization was also hypercellular and showed an infiltration by macrophages, some of which showed erythrophagocytosis. However, by day 18 the marrow had become very hypocellular.

**Lymph nodes.** In patient 1, the lymph node architecture was distorted by a massive and diffuse proliferation of immunoblastic lymphocytes (Fig 2). Sinuses were open, though compressed. Only in focal areas was the superficial cortex preserved. The immunoblasts did not have plasmacytoid features. Touch preparations showed that the immunoblasts were large in size, had oval nuclei with multiple prominent nucleoli, and a moderate amount of blue cytoplasm. Their cytologic features were consistent with T immunoblasts seen in response to viral stimuli. The number of small lymphocytes were markedly diminished. Mononuclear phagocytes were present throughout the lymph node. Leukophagocytosis was present and rare erythrophagocytosis was seen.

In patient 2, the lymph node showed a diffuse infiltration by benign-appearing mononuclear phagocytes (Fig 3). Sinuses were open, though compressed. Scattered immunoblasts were present throughout the node. Focal necrosis was seen. Germinal centers were not present. The macrophages infiltrating the lymph nodes, bone marrow, and liver in both patients were cytologically benign. They had low nuclear-cytoplasmic ratios, large folded nuclei, and abundant pink cytoplasm. Erythrophagocytosis and leukophagocytosis were easily seen.
Liver. The liver biopsy from patient 2 performed on day 8 showed large portal infiltrates composed of small lymphoid cells, lymphoblasts, and macrophages. The liver also showed a moderate degree of fatty metamorphosis.

Virological Studies

Acute EBV infection was well documented in each case by serology and demonstration of virus-infected lymphocytes (Table 2). Both patients developed the characteristic antibody responses observed in the acute phase of EBV infection: IgM and IgG-VCA in the absence of EBNA antibodies. Patient 1 had easily demonstrable EBNA-positive cells in a lymph node biopsy, and a spontaneous EBV-infected lymphoblastoid cell line was established from the bone marrow aspirate. Patient 2 had electron microscopy performed on a lymph node and liver biopsy (Fig 1), which demonstrated many lymphocytes with intracellular virus particles consistent by size and morphology with EBV.

Results of the modified Eckhardt gel technique are shown in Fig 4. A positive control, the permissively infected Burkitt's lymphoma derived cell line P3HR-1, demonstrated both circular (C) and linear (L) EBV-DNA (lane I). A spontaneous lymphoblastoid cell line obtained from patient 1 was applied to the vertical gel, and both circular and linear EBV-DNA were visualized (lanes 2 and 3). When lymph node tissue, snap-frozen in liquid nitrogen, was applied to the vertical
EBV-ASSOCIATED HEMOPHAGOCYTIC SYNDROME

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Hospital, day 2</th>
<th>1:80</th>
<th>1:160</th>
<th>1:20</th>
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<td></td>
<td>Hospital, day 14</td>
<td>1:80</td>
<td>1:160</td>
<td>1:20</td>
<td>&lt;1:5</td>
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<tr>
<td>Patient 2</td>
<td>Hospital, day 5</td>
<td>1:40</td>
<td>1:40</td>
<td>1:40</td>
<td>&lt;1:5</td>
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<tr>
<td></td>
<td>Hospital, day 42</td>
<td>1:20</td>
<td>1:80</td>
<td>1:20</td>
<td>1:5</td>
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</table>

**Immunological Studies**

**Lymphocyte surface markers** (Table 3). Peripheral blood studies showed that both patients had normal numbers of total T lymphocytes. Enumeration of helper/inducer and cytotoxic/suppressor T lymphocyte subsets revealed relatively normal percentages of T helper/inducer cells with an increased percentage of cytotoxic/suppressor cells. This resulted in a decreased helper-suppressor ratio. Increased numbers of Leu-7 positive cells were found in the single patient studied with this monoclonal antibody. Patient 1 had lymphocyte markers performed on the lymph node biopsy obtained on day 5 of hospitalization. Most lymph node cells expressed T cell antigens. B cells were not found by staining with antibodies against IgM or IgD chains. The majority of the T cells were activated as indicated by their expression of HLA-DR, common determinants (46%, p23, 30 positive). These T cells were expanded in lectin-free interleukin 2 (IL-2). After several days in culture, 43% expressed the OKT₆ cytotoxic/suppressor antigen, and 19% expressed the OKT₆ helper/inducer antigen.

**Lymphocyte Proliferation Studies** (Table 4)

In vitro proliferation of lymphocytes in the presence of PHA, Con A, and PWM was markedly depressed on day 6 in patient 2, whereas patient 1, who was studied on day 16 only, showed a significant depression of response to Con A. Early studies of patient 1 could not be performed because of the paucity of lymphocytes resulting from anemia and pancytopenia. Patient 2 had normal lymphocyte proliferation responses to the three T cell mitogens by day 35 of hospitalization.

**Cytotoxicity Studies** (Table 5)

Patient 1 was studied for cell-mediated cytotoxicity against a variety of target cells. NK cell activity against the K562 and Daudi target cells was normal, and activity could be augmented by preincubation of effector cells in α-interferon. Patient 1 did not demonstrate significant killing of the EBV-infected B lymphoblastoid cell line.
phoblastoid cell line 8392 (7% specific immune release).

**DISCUSSION**

Since the original description of VAHS by Risdall in 1979,1 little new information regarding the pathogenesis of this syndrome has been provided. Although five of the patients in the original report by Risdall had no known underlying immune deficiency, 14 patients were presumably immunosuppressed as a result of azathioprine and prednisone therapy for systemic lupus erythematosus (one patient) or renal transplantation (13 patients).1 However, immunological studies in patients experiencing VAHS have not been previously reported. It is noteworthy that six of the patients reported by Risdall1 and the single patient with EBV-associated VAHS reported by Wilson et al4 succumbed to their infections, suggesting an inadequate immune response to the invading viral agent.

In the two patients reported here, we have documented transient immunological abnormalities that may be helpful in the differential diagnosis of this potentially confusing syndrome. Both patients demonstrated abnormal lymphocyte proliferative responses to T cell-dependent mitogens. Such findings are commonly seen in individuals who are experiencing acute EBV-induced infectious mononucleosis.16 There was only a mild decrease in the ratio of T helper/inducer cells to T cytotoxic/suppressor cells (1.0 and 1.2). These ratios are usually lower in the acute phase of infectious mononucleosis and during overwhelming EBV infection, as seen in the X-linked lymphoproliferative syndrome.13 Neither of our patients showed many of the atypical lymphocytes characteristic of acute EBV infection. During acute EBV-induced infectious mononucleosis, these atypical cells primarily represent activated cytotoxic/suppressor T cells that are functionally active in lysing EBV-infected B lymphocytes and suppressing EBV-induced polyclonal B cell activation.17 The paucity of atypical activated cytotoxic T cells in the peripheral blood in patient 1 is evidenced by the absence of cytotoxicity for an EBV-infected, B lymphoblastoid cell line normally lysed by patients with acute infectious mononucleosis.14 This finding further distinguishes EBV-associated hemophagocytic syndrome from XLP, in which marked killing of both EBV-infected and uninfected target cells is observed.11

The pathology of VAHS has been somewhat controversial, with regard to its differentiation from malignant histiocytosis (also termed histiocytic medullary reticulosis).1,18,19 Risdall et al found that the major criteria distinguishing VAHS from malignant histiocytosis are the presence of cytologically neoplastic histiocytes and the relatively infrequent cytoplasmic basophilia that is observed in malignant histiocytosis.1 Subsequent reports indicate that this differentiation is not always obvious.2,5 In malignant histiocytosis, the

<p>| Table 3. Lymphocyte Surface Markers in Virus-Associated Hemophagocytic Syndrome |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Lymphocyte Source</th>
<th>B Lymphocytes</th>
<th>T Lymphocytes</th>
<th>OKT8 (Cytotoxic)</th>
<th>OKT4 (Helper/Inducer)</th>
<th>Leu-7 (HNK-1 Natural Killer)</th>
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</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Peripheral blood, day 3</td>
<td>16</td>
<td>71</td>
<td>46</td>
<td>46</td>
<td>1.0</td>
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<td>Lymph node, day 5</td>
<td>0</td>
<td>61</td>
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<td>ND</td>
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<tr>
<td>Peripheral blood, day 16</td>
<td>2</td>
<td>83</td>
<td>66</td>
<td>34</td>
<td>1.9</td>
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<td>Patient 2</td>
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<tr>
<td>Peripheral blood, day 6</td>
<td>13</td>
<td>72</td>
<td>67</td>
<td>54</td>
<td>1.2</td>
</tr>
<tr>
<td>Peripheral blood, day 35</td>
<td>0</td>
<td>75</td>
<td>53</td>
<td>38</td>
<td>1.4</td>
</tr>
<tr>
<td>Infectious mononucleosis (n = 13)</td>
<td>ND</td>
<td>76</td>
<td>31 ± 3.5</td>
<td>33 ± 3.8</td>
<td>0.96 ± 0.17</td>
</tr>
<tr>
<td>X-linked lymphoproliferative syndrome (n = 1) (blood)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Normal controls (n = 15)</td>
<td>10.5 ± 0.7</td>
<td>65.5 ± 7.9</td>
<td>48.8 ± 6.5</td>
<td>26 ± 6.0</td>
<td>1.98 ± 0.79</td>
</tr>
</tbody>
</table>

ND, not done.

| Table 4. Lymphocyte Proliferation Studies in Virus-Associated Hemophagocytic Syndrome |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|
| Mitogen                         | Day 16 | Laboratory JLS | Day 6 | Day 35 | Laboratory JHS |
| PHA                             | 73,662*| 71,450–82,414   | 18,537| 68,325  | 55,000–246,000 |
| Con A                           | 8,369  | 81,847–106,144  | 1,775 | 69,458  | 18,000–225,000 |
| PWM                             | 20,072 | 27,164–38,194   | 4,688 | 47,893  | 14,000–114,000 |

*Results are expressed as mean counts per minute of triplicate cultures that showed maximal stimulation to graded doses of mitogens.
malignant histiocytes pack the lymph node sinusoids and/or diffusely efface the lymph node architecture. Patient 1 demonstrated almost complete effacement of the lymph node architecture by a marked proliferation of immunoblasts. Immunoblastic proliferation has been noted by others in VAHS, although not to the extent observed in this patient. The lymph node biopsy from patient 1 could have been mistaken for malignant lymphoma because of the diffuse immunoblastic proliferation. Immunoblasts that lack plasmacytoid features in a patient with EBV infection suggest a reactive cell of T lymphocyte origin. This was confirmed by cell surface markers. It appears that both helper/inducer and cytotoxic/suppressor T cell subpopulations were proliferating, as determined by cell surface staining after expansion of these cells in vitro. As reported by Risdall et al., occasional erythrophagocytosis and leukophagocytosis was observed.

Patient 2 demonstrated the more typical lymph node findings supposedly characteristic of VAHS. Infiltration by benign-appearing histiocytes was present throughout the lymph node. Occasional erythrophagocytosis and leukophagocytosis and small numbers of immunoblasts were noted. Wilson et al. reported that the bone marrow of their patient was hypercellular, whereas in the report by Risdall et al., hypocellularity was found in 17 of 19 patients. Both of our patients had hypercellularity early in their disease, but a repeat marrow in patient 2 performed three weeks later revealed hypocellularity. A liver biopsy in patient 2 revealed large portal infiltrates with lymphocytes, immunoblasts, and histiocytes. The lymphohistiocytic infiltration appeared more exuberant than that described by Risdall et al. There was also a small degree of fatty infiltration. This liver pattern is consistent with that reported in patients experiencing acute EBV-induced infectious mononucleosis. In most patients with acute EBV infection, the lymph nodes show follicular hyperplasia and expansion of the paracortex, where numerous immunoblasts may be found. In these patients with VAHS, the follicular hyperplasia is absent and the nodes show a diffuse increase in macrophages (patient 2, Risdall et al., Wilson et al.) or an immunoblastic proliferation that distorts the normal lymph node architecture. These pathologic abnormalities in conjunction with the functional abnormalities described in this report are consistent with an aberrant immune response to EBV.

Immunodeficiency or immunological immaturity may play a role in the pathogenesis of VAHS. EBV is a potent stimulator of the immune system and requires a complex interaction of immunoregulatory cells for host recovery. Thus, this virus can provoke an intense immunoblastic proliferation, which may result in cytokine production with activation of histiocytes. We have demonstrated what appears to be an immunoregulatory cell imbalance in Epstein-Barr VAHS. Atypical lymphocytes were notably diminished in the peripheral blood in the face of an intense T cell immunoblastic proliferation in the lymph node. Cytotoxic T cells that normally lyse EBV-infected B cells were also absent from the peripheral circulation. Further studies of immunoregulation in patients with EBV-induced VAHS are needed to confirm these observations.

Last, both patients with VAHS described here showed evidence suggesting a permissive infection with EBV (permissive infection refers to cells in which infectious virus is actively replicated: nonpermissive infection refers to cells in which viral genome is present and is replicated, but in which infectious virus particles are not produced). Acyclovir (9-2-[2-hydroxyethoxymethyl] guanine) inhibits linear EBV-DNA synthesis, limiting infectious virus production. Acyclovir has no effect on EBV-induced transformation of B lymphocytes in vitro and does not interfere with the
replication of EBV circular genomes. We have previously reported acyclovir not to be effective in limiting the EBV-induced B lymphocyte proliferation that occurs in men with the X-linked lymphoproliferative syndrome. Recently, we have demonstrated that EBV infection in XLP is nonpermissive, explaining the lack of response to acyclovir. Both patients with EBV-induced VAHS were treated with acyclovir, and each recovered uneventfully without recurrences. These observations along with the virological evidence of permissive EBV infection suggests that EBV-induced VAHS is associated with increased replication of Epstein-Barr virus in infected B lymphocytes or other lymphoreticular cells. Acyclovir may be efficacious in the treatment of such infections.

ACKNOWLEDGMENT

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REFERENCES

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