Epstein-Barr Virus–Associated Hemophagocytic Lymphohistiocytosis in Adults Characterized by High Viral Genome Load within Circulating Natural Killer Cells

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Epstein-Barr virus (EBV)–associated hemophagocytic lymphohistiocytosis (HLH) is a rare and aggressive disease usually encountered in the context of primary EBV infection. In most analyzed cases, EBV has been found predominantly in T cells. We describe the novel finding of high EBV genome numbers within circulating natural killer cells in adult patients with EBV-HLH.

Epstein-Barr virus (EBV) is a ubiquitous and predominantly B lymphotrophic human γ-herpesvirus. EBV infection of natural killer (NK) and T cells is likely to be a rare event and is strongly associated with a spectrum of lymphoproliferations characterized by the pathognomonic presence of monoclonal EBV in the T and/or NK cells: EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH), chronic active EBV infection, aggressive NK cell leukemia, and extranodal (nasal) T/NK cell lymphoma [1–3].

EBV-HLH is a clinicopathological syndrome encompassing a dramatically dysregulated immune response and hypercytokinemria, characterized clinically by fever, splenomegaly, and cytopenia accompanied by histological evidence of hemophagocytosis, resulting in extremely high serum levels of ferritin, lactate dehydrogenase, and soluble CD25. Without early and effective therapy, EBV-HLH has a high mortality rate, frequently due to multiorgan failure. Recently established diagnostic and therapeutic guidelines have contributed to improvements in survival rates [4]. Most descriptive reports of EBV-HLH have arisen from study centers in east Asia, with the majority focused on primary EBV infection in children or adolescents [5]. In contrast, hemophagocytic syndrome in adults usually has underlying causes other than EBV infection [6]. Of the rare cases of adult EBV-HLH reported, the majority have been in individuals of east Asian origin [7], although isolated cases arising in adults of other ethnicities have been described [8, 9].

Previous studies of EBV-HLH that have incorporated analysis of both tissue biopsies and circulating lymphocyte subsets have revealed that CD3⁺ T cells (usually the CD8⁺ subset) are the predominant infected cell type, with isolated cases that suggest less frequent coinfection of CD16⁺ cells [3]. This contrasts markedly with chronic active EBV infection, in which the presence of EBV in NK cells occurs in approximately one-half of cases [10]. In this study, we investigated adult patients who presented in the United Kingdom with EBV-HLH and describe a hitherto unreported finding that CD56⁺CD3⁻ NK cells were the predominant infected cell type in the peripheral blood.

Three adult patients with suspected EBV-HLH (mean age, 44 years) were referred to our institution for diagnostic investigations during the period 2007–2009. Anonymized clinical data were provided retrospectively by the referring clinicians, in accordance with ethics approval from the West Midlands Research Ethics Committee. All 3 patients had EBV loads of 10⁷–10⁸ genomes/mL in whole blood and fulfilled established clinical and laboratory diagnostic criteria for the diagnosis of EBV-HLH [4], including histological evidence of hemophagocytosis (Figure 1A). There were no preexisting clinical features indicative of a background of chronic active EBV infection; for example, none had skin lesions or synovitis. Table 1 summarizes the clinical and laboratory characteristics.

Patient 1 was a previously fit and healthy 60-year-old white man who presented with a 3-week history of fevers and lethargy. Hemophagocytosis was demonstrable in a second diagnostic bone marrow biopsy sample. Despite the administration of a variety of immunosuppressive therapies, only a transient response was achieved, and he died of hepatic failure 5 weeks after initial presentation.

Patient 2 was a 50-year-old white man with a history of ulcerative colitis who described several weeks of fevers, anorexia, and weight loss. He subsequently developed respiratory failure, which necessitated mechanical ventilation; chest radiography showed pulmonary infiltrates. A clinical response after therapy with a modified HLH-2004 protocol [4] was transient,

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and the patient died of respiratory failure 2 months after initial presentation.

Patient 3 was a previously healthy 21-year-old man of Arab origin who presented with a 4-week history of fever, sweats, weight loss, and cough and required intensive care support. His case fulfilled the diagnostic criteria for EBV-HLH, and a subsequent biopsy of a small lymph node confirmed coexisting classical Hodgkin lymphoma, with the presence of EBV-encoded RNA–expressing Reed-Sternberg cells. The HLH responded well to initial treatment with etoposide and dexamethasone, after which he was treated with a standard chemotherapy protocol for Hodgkin lymphoma.

To isolate lymphocyte subsets from blood, peripheral blood mononuclear cells (PBMCs) costained with phycoerythrin-Cy5-conjugated anti-CD19 or CD20, fluorescein isothiocyanate–conjugated anti-CD3, and phycoerythrin-conjugated anti-CD16 or anti-CD56 (all monoclonal antibodies 1:20; AbD-Serotec) were simultaneously sorted on a MoFlo cell sorter (Figure 1B).

EBV genome loads were assayed in triplicate with use of real-time polymerase chain reaction (PCR) for BALF5 DNA and...
normalized against cellular β2-microglobulin DNA, as described elsewhere [11]. EBV latent and lytic cycle gene transcripts Qp, Cp, Wp, LMP1, LMP2A, BZLF1, gp350, and EBV-encoded RNA were assayed with use of quantitative reverse-transcriptase PCR using previously published primer-probe combinations [11].

The cell sorting strategy and viral load findings in peripheral lymphocyte populations were as follows. For patient 1, we used CD16 as a peripheral blood NK cell marker (gating on CD16+CD3−CD19− cells) but, somewhat surprisingly, found the highest viral load in a population of small lymphocytes negative for CD16, CD3, and CD19. We hypothesized that this population may represent CD56brightCD16− NK cells, which are a minority (~10%) NK cell subset in normal peripheral blood [12]. Consequently, we used CD56 as an alternative NK cell marker for the subsequent 2 patients. Figure 1C shows the high numbers of viral genomes in the NK cell populations, compared with B and T cells. In all 3 cases, the number of CD56+CD3−CD19− cells (or CD16+CD3−CD19− cells in patient 1) as a proportion of total PBMCs was not increased (2%–5%). CD3+ lymphocytes constituted the majority population, and all patients were relatively B lymphopenic (~2%).

The available cell numbers restricted analysis of EBV gene expression to patients 1 and 2. EBV gene expression analysis was performed on total PBMCs; nonetheless, we interpret the data as meaningful, because patient 1’s PBMCs were devoid of B cells, and for patient 2, the viral load in the NK cell fraction was greater than tenfold higher than in the B cells. Reverse-transcriptase quantitative PCR assays specific for viral antigen-encoding transcripts (Wp, Cp, Qp, LMP1, LMP2, and BZLF1) all produced negative results, although the EBV-encoded RNA transcripts were expressed in both cases and formally quantified in patient 1, whose levels exceeded those seen in an EBV-transformed B cell (Figure 1D).

We believe that this is the first report to demonstrate high levels of EBV in circulating CD56+ lymphocytes in adult patients with EBV-HLH. A previous study that separated peripheral blood lymphocytes to identify the EBV-infected cell type relied on a semiquantitative assessment with use of EBV-encoded RNA in situ hybridization [3] and, consistent with the majority of reports, revealed CD8+ T cells as the dominant target cell and fewer EBV-encoded RNA–positive CD16+ cells present. A recent study that adopted EBV-specific quantitative PCR investigated the phenotype of infected peripheral blood lymphocytes in non-Asian children with HLH and revealed the dominant viral load in CD19+ cells, followed by CD3+ T cells, but NK cells were excluded by their isolation protocol [13]. The lack of previous reports describing peripheral blood CD56+ NK cells as the dominant EBV target may be due to patient, epidemiological, or immune factors or to this cell population not having been specifically investigated before.

Because of limitations in the available data, viral gene expression in the context of EBV-HLH remains ill defined [14]. In 2 cases, we found an apparently tightly restricted pattern of viral gene expression with high levels of EBV-encoded RNAs exclusively expressed, but no protein-encoding transcripts were detected (Figure 1D). The importance of this observation is unclear, but it may be of relevance in light of a recent study that suggested a role for EBV-encoded RNA, present in serum samples from patients with HLH, in disease pathogenesis [15]. EBV-HLH is most commonly described in the context of primary infection with EBV, but the 3 adults that we describe had (in common with more than 90% of adults worldwide) serological evidence of prior EBV infection. Whether acquired immune dysfunction is required to trigger the onset of EBV-HLH in EBV-immune adults is not clear. Although EBV-HLH has been described in the context of immunosuppression [16], most patients are apparently immunocompetent. Among our cases, patient 1 had no history to suggest immune dysfunction, patient 2 was receiving therapy with 5-aminosalicylic acid (mesalazine), and patient 3 subsequently received a diagnosis of

Table 1. Clinical and Laboratory Characteristics of Adult Patients with Epstein-Barr Virus (EBV)–Associated Hemophagocytic Lymphohistiocytosis (HLH)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Splenomegaly and pancytopenia</th>
<th>Serum ferritin level, μg/L</th>
<th>Serum LDH level, IU/mL</th>
<th>HLH criteria met</th>
<th>EBV serological characteristics</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>&gt;16,500</td>
<td>5,602</td>
<td>6 of 8</td>
<td>IgM VCA</td>
<td>IVIG, rituximab, ganciclovir, dexamethasone, cyclosporin A</td>
<td>Died</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>22,699</td>
<td>1,879</td>
<td>7 of 8</td>
<td>+</td>
<td>Methylprednisolone, IVIG, etoposide, cyclosporin A</td>
<td>Died</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>4,203</td>
<td>2,085</td>
<td>6 of 8</td>
<td>+</td>
<td>Dexamethasone, etoposide</td>
<td>Complete response</td>
</tr>
</tbody>
</table>

**NOTE.** EA, early antigen; EBNA, EBV nuclear antigen; IVIG, pooled human immunoglobulins; LDH, lactate dehydrogenase; ND, not detected; VCA, viral capsid antigen; +, positive.

a Plus sign indicates antibody detected by means of enzyme immunoassay and/or immunofluorescence. Ellipses indicate characteristics not assessed.
b Henter et al [4].
EBV-positive Hodgkin lymphoma (mixed cellularity subtype), which has recently been recognized to be associated with HLH [17], although infection of NK cells in the context of EBV-positive Hodgkin lymphoma has not been previously described.

We have also been aware of 3 other cases of EBV-HLH in adults from the UK Midlands region during the period 2007–2009, 2 of which were associated with EBV-positive Hodgkin lymphoma in the context of human immunodeficiency virus infection. In these cases, the clinical features of HLH were quite distinct from those of Hodgkin lymphoma and were likely due to a coincident T or NK cell infection (as in patient 3), although we were unable to investigate these additional patients using the method described here. The remaining case was more typical, coinciding with primary EBV infection in a young adult.

The high mortality rate among patients with EBV-HLH is due, at least in part, to delays in diagnosis that result from the similarity between its initial clinical presentation and that of a wide range of infective and inflammatory conditions, which is often compounded by the apparent absence of hemophagocytosis on initial biopsy. Although EBV-HLH is a rare disease, we believe that the incidence in adults in Western countries is underestimated and, importantly, that EBV-HLH can occur in apparently EBV-immune individuals. The criteria established by Henter et al [4] remain a useful guide to diagnosis. Delineating the phenotype of the virus-harboring cell to inform the application of targeted therapies is an attractive concept but would necessitate larger collaborative studies of adults and children with EBV-HLH.

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References


