CLONAL KARYOTYPE ABNORMALITIES IN EBV-ASSOCIATED HEMOPHAGOCYTIC SYNDROME

JIANN-SHIUH CHEN,* CHING-CHERNG TZENG,° CHAO-JUNG TSAO, WU-CHOU SU,TSAI-YUN CHEN, YUN-CHIH JUNG,° IH-JEN SU°

*Departments of Pediatrics, °Medicine and Pathology, National Cheng Kung University Hospital, Tainan, Taiwan

ABSTRACT

Background and Objective. An EBV-associated hemophagocytic syndrome (HS) in previously healthy children or young adults has been documented in Taiwan. The exact nature of this syndrome, i.e., either an infectious process or a neoplastic disease, remains to be clarified.

Methods. Three patients diagnosed as having HS were studied retrospectively. Chromosomes from bone marrow were examined by a conventional trypsin-Giemsa banding technique and karyotyped at the beginning of diagnosis or during treatment. In situ hybridization studies for EBV using EBER1 were performed.

Results. All three patients presented the classic manifestations of HS including fever, splenomegaly, jaundice, pancytopenia and coagulopathy. Bone marrow aspiration revealed atypical lymphocyte and histiocyte infiltration with hemophagocytosis. EBV genomes were found in bone marrow in all patients. In addition to normal mitotic cells, clonally karyotypically abnormal cells were demonstrated in all three patients whose diseases were rapidly progressive and eventually refractory to etoposide-based therapy. The consistent karyotypical abnormality of add(9)(p24) was noted in two of them.

Interpretation and Conclusions. Although HS is usually considered a reactive process, the emergence of clonal cytogenetic abnormalities should be considered a malignant entity and treated with more intensive chemotherapy. A large series of cytogenetic and molecular studies is needed to clarify the exact nature of this fatal disease.

©1997, Ferrata Storti Foundation

Key words: hemophagocytic syndrome, Epstein-Barr virus, clonal karyotype abnormalities
Chromosome analysis

Cytogenetic studies were done on BM mononuclear cells from these three patients at the beginning of diagnosis (case #3) or during treatment (case #1: 69 days after diagnosis; case #2: 10 days and 38 days after diagnosis, respectively). The cells were harvested directly or after a 24-hour culture with or without synchronization using methotrexate. Metaphases were banded by a conventional trypsin-Giemsa banding technique\(^{15}\) and karyotypes were interpreted according to ISCN (1995).\(^{16}\)

Studies of EBV by EBER1 in situ hybridization

The in situ hybridization studies of EBV RNA were performed using a 30-bp oligonucleotide probe complementary to a portion of the EBER 1 gene, an actively transcribed region of EBV genome (up to 10 copies per cell in latently infected cells). Briefly, 10-µm sections cut from paraffin blocks of formaldehyde- or B5-fixed BM tissue were deparaaffinized, dehydrated, predigested with pronase, prehybridized, and hybridized overnight with a probe concentration of 0.25 ng/L. The B5-fixed tissue sections, pretreated with a 1% iodine xylene solution, were deparaffinized for 15 minutes to remove heavy metals. After washing, detection was accomplished using avidin-alkaline phosphatase conjugate followed by development of the signal with Mc Gadey’s substrate. A brown or blue-brown color within the nucleus over background levels was considered a positive reaction. A known EBV-positive neoplasm was used as a positive control, and an EBV-negative lymphoid tissue was used as a negative control in each run. Any slide negative for EBV RNA was tested for viability of total RNA using a poly d(T) probe.

Results

Clinical manifestations and laboratory data

The clinical manifestations of these patients are summarized in Table 1. All three patients were male and the mean age was 18 years. Fever was an initial symptom in all patients ranging from seven days to two weeks. Splenomegaly (in three out of three patients), hepatomegaly (in two out of three), pan-cytopenia (in three out of three), and ascites (in two out of three) were found at the beginning of diagnosis. Progressive neutropenia, thrombocytopenia and anemia were observed within a few days after presentation. Coagulopathy in the form of prolonged activated partial thromboplastin time was found in all three patients and hypofibrinogenemia was noted in two of the patients, one of which showed evidence of disseminated intravascular coagulopathy (DIC). All patients had deranged liver functions, as demonstrated by elevated transaminase, marked elevation of lactate dehydrogenase and direct hyperbilirubinemia. Elevation of the serum triglyceride level was observed in all patients as well.

Bone marrow findings

Bone marrow aspiration and histology in all patients revealed varying degrees of atypical lymphocyte infiltration and histiocyte proliferation with active hemophagocytosis. The mature-appearing histiocytes were characterized by a low nuclear/cytoplasmic ratio, mature chromatin and inconspicuous nucleoli with ingested erythrocytes, and platelet and/or nucleated marrow elements (Figure 1).

At the beginning, the section in case #1 showed moderate hypocellular marrow (40%), while normocellular and hypercellular marrow were found in case #2 (50%) and case #3 (70%), respectively. Both myeloid and erythroid series were hypoplastic in case #1, and the maturation of residual hematopoietic elements was insignificant.

The marrow histology, when cytogenetic studies were performed simultaneously, revealed moderate hypocellular marrow (40%) in case #1, hypercellular marrow (85% and 80%) in case #2 (twice) and hypercellular marrow (70%) in case #3. The atypical lymphocyte infiltration in the bone marrow

---

Table 1. Clinical characteristics in patients with EBV-containing hemophagocytic syndrome.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/Sex</th>
<th>Fever (≥ 7 D)</th>
<th>Splenomegaly (≥ 3 cm)</th>
<th>WBC/ANC (x10^9/L)</th>
<th>Hb (g/dL)</th>
<th>Plt (x10^9/L)</th>
<th>Triglyceride (mg/dL)</th>
<th>Fibrinogen (mg/dL)</th>
<th>EBER1 (BM)</th>
<th>Treatment regimens</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3Y/M</td>
<td>+</td>
<td>0.80/0.42</td>
<td>7.1</td>
<td>17</td>
<td>547</td>
<td>109</td>
<td>+</td>
<td>VP-16, IVIG, Steroid</td>
<td>2.5M</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17Y/M</td>
<td>+</td>
<td>0.90/0.39</td>
<td>9.5</td>
<td>10</td>
<td>221</td>
<td>300</td>
<td>+</td>
<td>VP-16, Steroid, CHOP</td>
<td>3M</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35Y/M</td>
<td>+</td>
<td>1.50/0.67</td>
<td>15.3*</td>
<td>15</td>
<td>334</td>
<td>82</td>
<td>+</td>
<td>Steroid</td>
<td>3D</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations. IVIG, Intravenous immunoglobulin; CHOP, Cyclophosphamide, Epirubicin, Vincristine, Prednisolone; WBC, white cell count; ANC, absolute neutrophil count; Hb, hemoglobin; Plt, platelet; BM, bone marrow; Y, year; M, month; D, day. *Post-transfusion at outside clinics.
accounted for 17% of all nucleated cells for case #1, 18% and 20% for case #2 (twice) and 20% for case #3. Reactive histiocytes were scattered and were usually less than 3% with hemophagocytosis.

Clinical course
Patient #1 was treated with intravenous immunoglobulin (IVIG), prednisolone and etoposide with a transient response. His disease became gradually refractory to similar treatment and the patient died of the progressive disease at two and half months after diagnosis.

Patient #2 had symptoms of epigastralgia and headache in addition to prolonged fever. His condition improved after steroid therapy. Unfortunately, fever flared up again one month later and his disease was refractory to steroid, etoposide and even modified CHOP regimens (cyclophosphamide, epirubicin, vincristine and prednisolone). He died at three months after diagnosis.

Patient #3 manifested sepsis-like symptoms and signs, and died soon thereafter in spite of vigorous supportive treatment and pulse methylprednisolone therapy.

Chromosome analysis
The results of BM cytogenetic analysis on these three cases of HS (four times, in all) are summarized in Table 2.

Clonal karyotype abnormalities were noted in all three cases. Seventeen of the 20 cells analyzed in patient #1 were abnormal. Two clonal abnormalities were noted, i.e. 47,XY, der(11) dup(11) (q23;q23)(q11)(q21;q23), del(17)(p13), add(19)(p13)[cp14]/47,XY, idem, add(19)(p13)[cp3] (Figure 2). Patient #2 underwent the cytogenetic study twice. The first time, the study was completely normal. In the second study, however, five of the 20 cells had abnormal chromosomes. The only clonal abnormalities were 45,X,-Y, add(9)(p24), add(17)(p11)[cp5] (Figure 3). The karyotypes of patient #3 were normal in only 3 of the 20 cells examined, and the clonal abnormalities were 46,XY, add(4)(q31), add(7)(q22), add(9)(p24)[cp17] (Figure 4). It is
worth noting that add(9)(p24) was consistently detected in cases #2 and #3.

**EBV genomes in BM tissues**

The EBV RNA was detected in the bone marrow biopsies of all three cases by *in situ* hybridization using an antisense EBER1 probe in nuclei of transformed lymphocytes (data not shown).

**Discussion**

We presented here the cytogenetic studies of three cases of EBV-associated HS. All three cases revealed clonal karyotype abnormalities, suggesting that the HS in these patients represents a clonal or neoplastic process. For many years, there has been controversy over the exact nature of these EBV-associated HS, i.e. whether it belongs to an infectious process or a neoplastic disease. The HS in toddlers is generally regarded as a fatal form of infectious mononucleosis, whereas in the adult type, an EBV-containing T-cell lymphoma presenting HS behaves like malignant histiocytosis. Our studies on one young child (3 years old) and two young adults revealed clonal karyotype abnormalities, suggesting a neoplastic process in all 3 cases. The results were consistent with the previous studies of a clonal proliferation of EBV genomes in these patients.

In these fatal EBV-associated HS, the EBV has been shown to infect T lymphocytes, as opposed to B-cells, as was conventionally believed. In the past decade, the treatment of HS has improved greatly with the advent of the etoposide-based regimen. Others applied more intensive treatment with allogeneic bone marrow transplantation in such patients, which obtained successful results in limited cases. A preliminary trial of immunomodulation incorporating IVIG and etoposide has been adopted to combat the childhood cases with dramatic response. However, the long-term follow-up of these childhood cases revealed a relapsing and a progressive course with subsequent development of tumors in certain patients (Su et al., unpublished observation). These observations suggest that a clonal proliferation may have already prevailed. The current data of clonal karyotype abnormalities in our patients support such an assumption.

Whether the clonal proliferation already exists at presentation or develops during clinical progression remains to be clarified; based on the observations of Kaneko et al. and on our current cytogenetic data it appears that both possibilities exist.

**Table 2. Bone marrow cytogenetic findings in three patients of EBV-containing hemophagocytic syndrome**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex</th>
<th>No. of metaphase</th>
<th>Karyotypes of abnormal clones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td>1</td>
<td>3Y/M</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>17Y/M</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>35Y/M</td>
<td>3</td>
<td>17</td>
</tr>
</tbody>
</table>
In the 9 cases of hemophagocytic lymphohistiocytosis reported by Kaneko et al., chromosomal abnormalities were demonstrated in six subjects; clonally karyotypically abnormalities were detected in two patients, but no consistent abnormalities were reported. The presence of chromosomal abnormalities is correlated to an aggressive disease while a normal chromosomal analysis represents a benign course. In the three cases we analyzed, case 1 and case 3 already had clonal karyotype abnormalities at presentation. The karyotype analysis was normal at presentation for case 2, however, and clonal karyotype abnormalities were detected only four weeks later, showing the same abnormalities as case 3 – add(9)(p24). This suggests that breakpoint at 9p24 is probably associated with the disease progression.

With the emergence of karyotypically abnormal clones, whether in the beginning or during the course, we suggest that HS be considered a neoplastic process. And, in addition to the immunomodulation regimen to combat the reactive hemophagocytic process and/or the cytokines storm effect, more intensive chemotherapy than that used currently should be applied to such patients. Any efforts to document the clonal entity should not be overlooked in the face of EBV-containing HS. Series and repeated chromosome studies of bone marrow or tissues are recommended in cases of HS in order to detect the clonal changes and apply the best treatment strategy early on. Finally, a large series of cytogenetic studies are needed to clarify the exact nature of this fatal disorder.

References