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

Hemophagocytic syndrome (HS) is a histiocytic reactive process frequently associated with infection (bacterial or viral)[1-4] or malignancies.[5,6] This syndrome is characterized by fever, hepatosplenomegaly, pancytopenia, hypertriglyceridemia and/or hypofibrinogenemia, and a florid histiocytic proliferation with hemophagocytosis in the bone marrow (BM), spleen and lymphoid system.[7] Recently, an EBV-associated HS has been demonstrated in children or young adults.[8] The EBV infects T lymphocytes in this unique entity.[9,10] Currently, the cytokines such as tumor necrosis factor-α, interferon-γ, and interleukin-1β released from the EBV-infected T-cells or macrophages have been presumed to be implicated in the pathogenesis of HS.[11,12]

A major diagnostic dilemma for this syndrome is the distinction between reactive EBV-infected T cell proliferation and EBV-containing T cell lymphoma, since both diseases share common clinical manifestations. Although clonal proliferation of the EBV genome has been applied to resolve this issue,[13] a clonal marker based on the lineage of the host cells is required. This effort is important since the principle of treatment is mainly based on the nature of underlying diseases. In this study, karyotypic chromosomal analysis was performed in three patients initially presenting HS. All three cases were confirmed as having clonal chromosomal abnormalities and were associated with EBV infection by *in situ* EBER1 hybridization.

Patients and Methods

Patients

In the National Cheng Kung University Hospital in 1995 three patients were diagnosed as HS, and fulfilled the diagnostic criteria proposed by the Histiocyte Society.[14] No associated malignancies or underlying immunodeficiency had been found previously. The clinical records and laboratory data were reviewed and analyzed.

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-Giema 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 deparaffinized, 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), pancytopenia (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/cytoplasmatic 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 normocelluar 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 unsignificant.

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.

Case no.	Age/ Sex	Fever (≥ 7 D)	Spleno- megaly (≥ 3 cm)	Cytopenia			Triglyceride (mg/dL)	Fibrinogen (mg/dL)	EBER1 (BM)	Treatment regimens	Survival
				WBC/ANC (x10⁹/L)	Hb (gm/dL)	Plt (x10⁹/L)					
1	3Y/M	+	+	0.80/0.42	7.1	17	547	109	+	VP-16, IVIG, Steroid	2.5M
2	17Y/M	+	+	0.90/0.39	9.5	10	221	300	+	VP-16, Steroid, CHOP	3M
3	35Y/M	+	+	1.50/0.67	15.3*	15	334	82	+	Steroid	3D

*Abbreviations. IVIG, Intravenous immunoglobulin; CHOP, Cyclophosphamide, Epirubicin, Vincristine, Prednisolone; WBC, white cell count; ANC, absolute neutrophil count; Hb, hemoglobin; Pl, platelet; BM, bone marrow; Y, year; M, month; D, day. *Post-transfusion at outside clinics.*
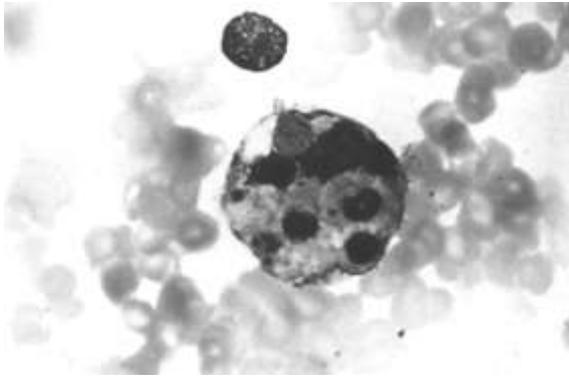
Figure 1. The bone marrow aspirated smear of case #1 at initial presentation showing one histiocyte with active hemophagocytosis. Notice the low nuclear:cytoplasmic ratio, mature chromatin pattern and inconspicuous nucleoli (Liu's stain, original magnification ×1000).
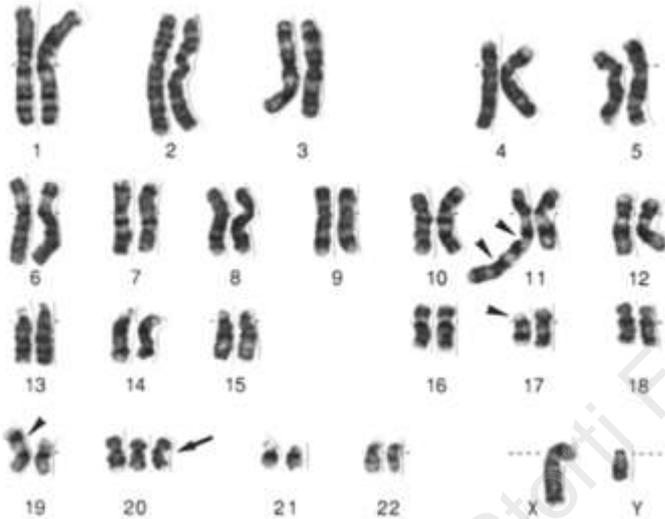

Figure 2. Karyotype from case #1 showing 47, XY, der(11)dup(11)(q23;q23q11)t(5;11)(q21;q23), del(17)(p13), add(19)(p13), +20.
Arrows indicate rearranged chromosomes.

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;q23q11)t(5;11)(q21;q23), del(17)(p13), +20 [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
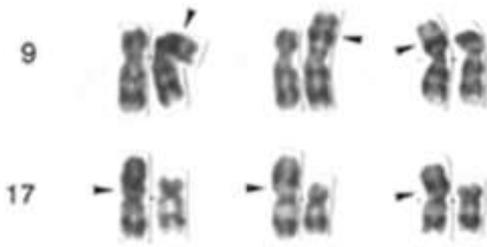
Figure 3. Partial karyotype of the clonal abnormalities from case #2 showing add(9)(p24), add(17)(p11). Arrows indicate rearranged chromosomes.

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,[8,17] whereas in the adult type, an EBV-containing T-cell lymphoma presenting HS behaves like malignant histiocytosis.[6] 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.[13,18]
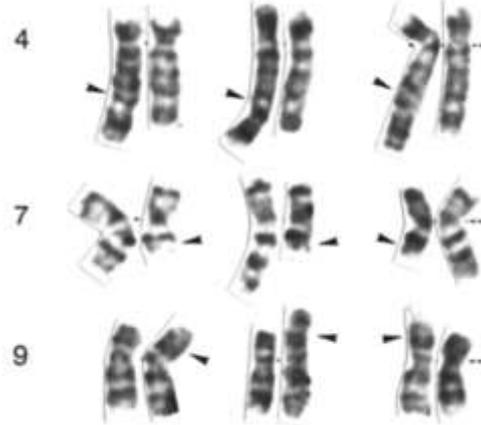

Figure 4. Partial karyotype of the clonal abnormalities from case #3 showing add(4)(q31), add(7)(q22), add(9)(p24). Arrows indicate rearranged chromosomes.

In these fatal EBV-associated HS, the EBV has been shown to infect T lymphocytes, as opposed to B-cells, as was conventionally believed.[10] In the past decade, the treatment of HS has improved greatly with the advent of the etoposide-based regimen.[19] Others applied more intensive treatment with allogeneic bone marrow transplantation in such patients, which obtained successful results in limited cases.[20] A preliminary trial of immunomodulation incorporating IVIG and etoposide has been adopted to combat the childhood cases with dramatic response.[21] 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 abnormalites 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.*[18] 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

Case No.	Age/Sex	No. of metaphase		Karyotypes of abnormal clones
		Normal	Abnormal	
1	3Y/M	3	17	47, XY, der(11)dup(11)(q23;q23q11)t(5;11)(q21;q23), del(17)(p13), +20 [cp14] / 47, XY, idem, add(19)(p13) [cp3]
2	17Y/M	20	0	
		15	5	45, X, -Y, add(9)(p24), add(17)(p11) [cp5]
3	35Y/M	3	17	46, XY, add(4)(q31), add(7)(q22), add(9)(p24) [cp17]

In the 9 cases of hemophagocytic lymphohistiocytosis reported by Kaneko et al.,[18] chromosomal abnormalities were demonstrated in six subjects; clonally karyotypical abnormalilities 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

1. Reisman RP, Greco MA. Virus-associated hemophagocytic syndrome due to Epstein-Barr virus. Hum Pathol 1984; 15:290-3.

2. Risdall RJ, Brunning RD, Hernandez JI, Gordon DH. Bacteria-associated hemophagocytic syndrome. Cancer 1984; 54:2968-72.
3. Boruchoff SE, Woda BA, Pihan GA, Durbin WA, Burstein D, Blacklow NR. Parvovirus B19-associated hemophagocytic syndrome. Arch Intern Med 1990; 150:897-9.
4. Luppi M, Torelli G. The new lymphotropic herpesviruses (HHV-6, HHV-7, HHV-8) and hepatitis C virus (HCV) in human lymphoproliferative diseases: an overview. Haematologica 1996; 81:265-81.
5. Craig FE, Clare CN, Sklar JL, Banks PM. T-cell lymphoma and the virus-associated hemophagocytic syndrome. Am J Clin Pathol 1992; 97:189-94.
6. Su IJ, Hsu YH, Lin MT, Cheng AL, Wang CH, Weiss LM. Epstein-Barr virus-containing T-cell lymphoma presents with hemophagocytic syndrome mimicking malignant histiocytosis. Cancer 1993; 72:2019-27.
7. Balduini CL, Belletti S. A single cell summarizing the pathogenesis of cytopenias in hemophagocytic syndrome. Haematologica 1996; 81:190.
8. Chen RL, Su IJ, Lin KH, et al. Fulminant childhood hemophagocytic syndrome mimicking histiocytic medullary reticulosis. An atypical form of Epstein-Barr virus infection. Am J Clin Pathol 1991; 96:171-6.
9. Kawaguchi H, Miyashita T, Herbst H et al. Epstein-Barr virus-infected T lymphocytes in Epstein-Barr virus-associated hemophagocytic syndrome. J Clin Invest 1993; 92:1444-50.
10. Su IJ, Chen RL, Lin DT, Lin KS, Chen CC. Epstein-Barr virus (EBV) infects T lymphocytes in childhood EBV-associated hemophagocytic syndrome in Taiwan. Am J Pathol 1994; 144:1219-25.
11. Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in hemophagocytic syndrome. Am J Pediatr Hematol Oncol 1993; 15:92-8.
12. Akashi K, Hayashi S, Gondo H et al. Involvement of interferon-gamma and macrophage colony-stimulating factor in pathogenesis of haemophagocytic lymphohistiocytosis in adults. Br J Haematol 1994; 87:243-50.
13. Dolezal MV, Kamel OW, van de Rijn M, Cleary ML, Sibley RK, Warnke RA. Virus-associated hemophagocytic syndrome characterized by clonal Epstein-Barr virus genome. Am J Clin Pathol 1995; 103:189-94.
14. Henter JI, Elinder G, Ost A. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. The FHL Study Group of the Histiocyte Society. Semin Oncol 1991; 18:29-33.
15. Seabright M. A rapid banding technique for human chromosomes. Lancet 1971; 2:971-2.
16. Mitelman F. ISCN (1995): An International System for Human Cytogenetic Nomenclature. Basel: S. Karger; 1995.
17. Kikuta H, Sakiyama Y, Matsumoto S, et al. Fatal Epstein-Barr virus-associated hemophagocytic syndrome. Blood 1993; 82:3259-64.
18. Kaneko Y, Maseki N, Sakurai M et al. Clonal and non-clonal karyotypically abnormal cells in haemophagocytic lymphohistiocytosis. Br J Haematol 1995; 90:48-55.
19. Alvarado CS, Buchanan GR, Kim TH, Zaatari G, Sartain P, Ragab AH. Use of VP-16-213 in the treatment of familial erythrophagocytic lymphohistiocytosis. Cancer 1986; 57:1097-100.
20. Blanche S, Caniglia M, Girault D, Landman J, Griscelli C, Fischer A. Treatment of hemophagocytic lymphohistiocytosis with chemotherapy and bone marrow transplantation: a single-center study of 22 cases. Blood 1991; 78:51-4.
21. Chen RL, Lin KH, Lin DT, et al. Immunomodulation treatment for childhood virus-associated haemophagocytic lymphohistiocytosis. Br J Haematol 1995; 89:282-90.