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Case report

Virus-associated haemophagocytic syndrome with Epstein-Barr virus infection

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Summary. The clinical and histological findings of a 10-year-old girl with virus-associated haemophagocytic syndrome are presented. The serum levels of Epstein-Barr viral antigens were elevated. Epstein-Barr virus (EBV) genome was detected by polymerase chain reaction in bone marrow and lymph node specimens. Histologically, haemophagocytic histiocytes were present in bone marrow, and areas of non-suppurative necrosis were present in lymph nodes, where silver grain deposition of the EBV genome was demonstrated by in situ hybridization.

Key words: Virus-associated haemophagocytic syndrome – Epstein-Barr virus – In situ – Polymerase chain reaction

Introduction

The virus-associated haemophagocytic syndrome (VAHS) is a histiocytic proliferative disorder, with bone marrow and liver failure, for which the connection with a specific virus is often tenuous. Of the 19 patients described in the original report by Risdall et al. (1979), 2 patients were considered to have Epstein-Barr virus (EBV). McClain (1986) reported the presence of EBV-DNA in lymphocytes of patients with VAHS, and Reisman and Greco (1984) reported the presence of non-suppurative necrosis in lymph nodes. We present a case with non-suppurative necrosis in lymph nodes and with EBV genome shown by in situ hybridization. To the best of our knowledge, an in situ hybridization study has not been previously reported on patients with VAHS.

Case report

A 10-year-old Japanese girl was admitted to Kyushu Cancer Center Hospital. The history revealed that she had had hepatosplenomegaly at the age of 3 years, and was followed for chronic EBV infection due to high titres of EBV-associated antibodies in the serum. At presentation, she had a fever, hepatosplenomegaly, and ascites. Bone marrow aspirate and cervical lymph node biopsy were done. After the biopsy, she died with sepsis.

The laboratory findings on admission were as follows: haemoglobin 7.9 g/dl, haematocrit 24.1%, and red blood cell count $379 \times 10^4/mm^3$. The leukocyte count was $2,200/mm^3$, with 6% segmented neutrophils, 54% bands, 35% lymphocytes, and 5% monocytes. Atypical lymphocytes were not noted. The platelet count was $50,000/mm^3$, the erythrocyte sedimentation rate 1 mm/h, the prothrombin ratio 16.8/11.1, and the partial thromboplastin time 150 s. The total bilirubin was 1.9 mg/dl, alkaline phosphatase 712 units/l (normal, 130 units/l), asparate aminotransferase (SGOT) 346 units/l (normal, 37 units/l), lactate dehydrogenase 2,670 units/l (normal, 370 units/l); and ferritin 26,110 ng/ml (normal, 234 ng/ml).

The immunoglobulin levels were decreased: IgG 948 mg/dl (normal, 1,031 mg/dl), IgM 32 mg/dl (normal, 74 mg/dl), and IgA 63 mg/dl (normal, 115 mg/dl). In peripheral blood, the lymphocyte subpopulation determined by monoclonal antibodies was as follows: T3, 67.9%; T4, 59.9%; T8, 12.9%; T11, 75.0%; and B1, 6.6%. The pan-T-cell marker T3 was normal, but the number of suppressor cells as determined by T8 was decreased. This resulted in a markedly elevated T4:T8 ratio (>2). Mitogen responses to B- and T-cell stimulators were decreased: phytohaemagglutinin, 23,358 cpm (normal, 37,700), concanavalin A 12,424 cpm (normal, 24,300), and pokeweed mitogen 7,828 cpm.

Serological findings for EBV-related antibodies were as follows: the titre of IgM antibody to viral capsid antigen (VCA) was 1:10, IgG-VCA antibody, 1:10,240, early antigen-D component (EA-D) 1:160; and Epstein-Barr nuclear antigen (EBNA) 1:20.

Methods

For the polymerase chain reaction (PCR) the bone marrow aspirate and lymph node biopsy sections were deparaffinized and digested with protease K for 7 days. PCR for the *Bam*HI W region of the EBV genome (Saito et al. 1989; Ohshima et al. 1991) was done. Specific primers were synthesized on the published DNA sequence (primer 1: CCAGAGGTAAGTGGACTT; primer 2: GACCGGTGCCTTCTTAGG; probe: TTCTGCTAAGCCCAC), corresponding to the *Bam*HI W region of the EBV genome. The amplification was carried out with GeneAmp DNA amplification reagent kit and DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, Conn.). After 40 cycles of PCR amplification, we could detect spe-

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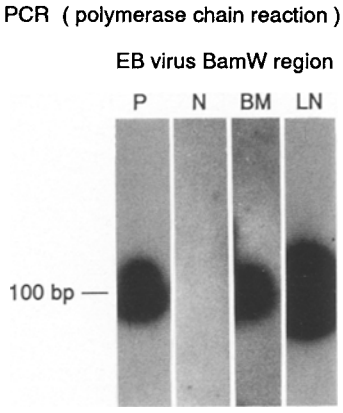

Fig. 1. Polymerase chain reactions (primer 1: CCAGAGGTAAGTGGACTT; primer 2: GACCGGTGCCTTCTTAGG; probe: TTCTGCTAAGCCCAC), corresponding to the *Bam*HI W region of the EBV genome, were performed with ^{32}P-labelled probe by the Southern blot method. *P*, Positive control; *N*, negative control; *BM*, bone marrow; *LN*, lymph node

cific bands of EBV in bone marrow and lymph nodes, using the Southern blot method (Fig. 1).

In situ hybridization studies were performed on paraffin-embedded lymph node tissue using a previously described method (Oshima et al. 1991). The tissue was hybridized with an EBV probe (EBV *Bam*HI V region; Enzo, Hudson, N.Y.), which had been radiolabelled with alpha ^{35}S dCTP by the random hexamer primer technique. The slides were washed. And autoradiographic exposure using an overlay of Konica NR-M2 emulsion (Tokyo, Japan) lasted 21 days. After development of the emulsion, the sections were counterstained with haematoxylin.

Results

Histological examination revealed the bone marrow to be hypercellular (Fig. 2). There was a leftward shift in the myeloid series, and megakaryocytes were increased in number. Numerous histiocytes were packed with erythrocytes and with numerous nucleated forms of either erythroid or lymphoid origin. Examination of the lymph node specimen revealed non-suppurative necrosis: some foci of necrosis with eosinophilic material, ghosts of cells, karyorrhectic debris, and foamy histiocytes, without neutrophils or plasmacytes in the cortex.

Dilated subcapsular, trabecular, and medullary sinuses were filled with histiocytes. Lymph follicles with germinal centres were preserved. The cells of non-suppurative necrosis reacted positively to the histiocytic markers KP1, Leu-M1 (Dakopatts, Glostrup, Denmark), alpha-1-antichymotrypsin, and lysozyme and positively to ferritin (Dakopatts), but negatively to the myeloid marker naphtol ASD acetate esterase (NASDA), T-cell marker UCHL-1 (Dakopatts), and the B-cell marker L26 (Kyowa Medix, Tokyo, Japan).

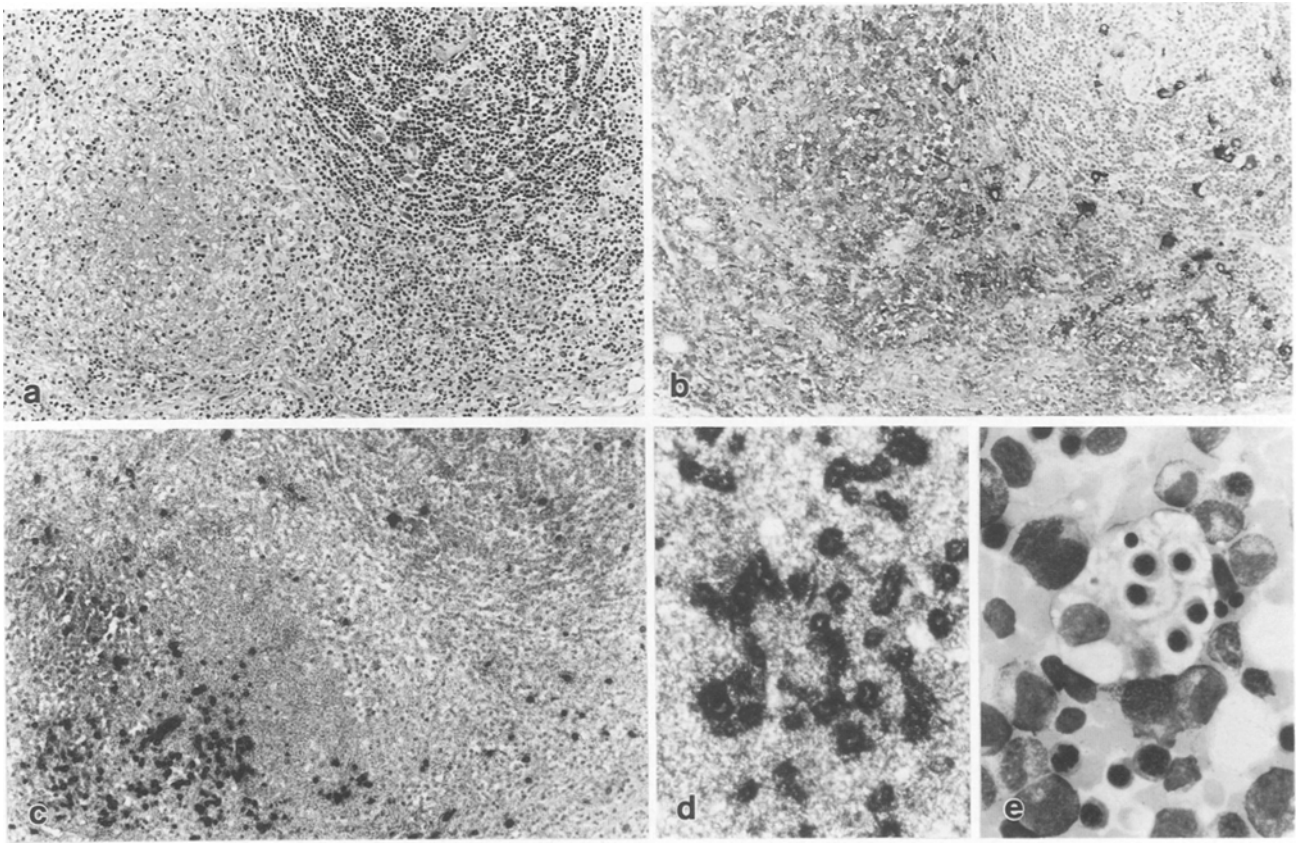

Fig. 2. **a** Non-suppurative necrosis and preserved lymph follicle are seen in the lymph node, ×100. **b** The cells in the non-suppurative necrosis show a positive reaction for ferritin, ×100. **c** In situ hybridization for EBV genomes. The deposition of grains is present in the cells of the non-suppurative necrosis, ×100. **d** ×400. **e** Phagocytosis is seen on a bone marrow smear. ×400

The in situ findings indicated the presence of EBV genome within the cells. The silver grains of EBV genome were probably in the histiocytes of the non-suppurative necrotic area, but not in the lymphocytes of lymph follicles. The fine distribution was not clear, because of the mechanical damage caused by the hybridization procedure. We used a specimen of non-specific lymphadenitis without EBV infection as the negative control, and the EBV silver grain deposits were not present, as reported previously (Ohshima et al. 1991).

Discussion

The patient reported here satisfies the criteria for VAHS as defined by Risdall et al. (1979): fever, hepatosplenomegaly with hepatocellular dysfunction, pancytopenia, and lymphadenopathy.

Non-suppurative necrosis in the lymph node of a patient with VAHS was previously observed by Reisman and Greco (1984). Lymph node necrosis has been reported in patients with the X-linked lymphoproliferative syndrome and other immunodeficiency states with EBV infection (Provisor et al. 1975; Purtilo et al. 1981). Also included in the differential diagnosis of non-suppurative necrosis of lymph node parenchyma are influenza (Tindle 1978), systemic lupus erythematosus (Rosai 1981), congenital herpes simplex viral infection (Krasznai and Gyory 1968), familial erythrophagocytic lymphohistiocytosis (Singer 1981), typical infectious mononucleosis (Tindle et al. 1972), and histiocytic necrotizing lymphadenitis (Kikuchi et al. 1986). Necrosis in the presence of lymphoid depletion rather than proliferation and haemophagocytic histiocytosis distinguishes VAHS from these other diseases, with the exception of familial erythrophagocytic lymphohistiocytosis, which may be morphologically indistinguishable from VAHS but differs in its clinical features.

A high serum ferritin level is important as a marker of VAHS (Esumi et al. 1988). According to in vitro studies, intracellular ferritin sharply increases during the maturation of monocytes into macrophages (Andreesn et al. 1984), and cultured monocytes in iron-containing medium or in the phagocytic process of erythrocytes accumulate ferritin rapidly (Worwood et al. 1984). In our patient, the level of serum ferritin was high and histiocytes of non-suppurative necrosis in the lymph node showed a positive reaction for ferritin.

Detection of antibodies to EBV-specific antigen (i.e. VCA, EA-D, and EBNA), permits the diagnosis of EBV infection in the absence of heterophile antibody or the typical features of infectious mononucleosis. Our patient's sera contained high titres of antibodies to IgG anti-VCA and EA-D, accompanied by a proportionately low titre of antibody to EBNA. Atypical patterns of antibodies to VCA and EA-D and delayed or impaired production of anti-EBNA have been observed in immunosuppressive states (Henle and Henle 1981). For example, in a report of a 5-year-old girl who died from prolonged EBV infection associated with hypergammaglobulinaemia and systemic immunoblastic proliferation, titres were as high as 1:160,000 for IgG anti-VCA and lower for anti-EBNA (Virelizier et al. 1978).

The fatal outcome of our patient might be due to the underlying immune deficiency, which was suggested by the decreased serum levels of IgG, IgM and IgA and the decreased numbers of suppressor T-cells of peripheral blood. McClain (1986) reported a fatal case of VAHS with the decreased suppressor T-cells and EBV infection. But in this case, the immunoglobulin levels were normal.

McClain et al. (1988) reported that 14 of 17 children with VAHS had EBV-DNA genome, as shown by DNA hybridization studies. We could not obtain fresh material to perform DNA hybridization, but EBV genome was detected by PCR and in situ hybridization.

Our in situ findings indicated the EBV genome within the histiocytes of the non-suppurative necrosis in the lymph node and the silver grains was not present in B-cell areas. EBV is a ubiquitous transforming herpes virus that replicates in oropharyngeal epithelial cells (Britton et al. 1978) and infects and mortalizes EBV-receptor-positive B-lymphocytes (Fingeroth et al. 1984), resulting in a polyclonal activation of B-lymphocytes in vitro (Epstein and Achong 1979; Cheeseman et al. 1980) and in vivo (Frizzera et al. 1981). B-cells must have small numbers of latent EBV genomes, but in our in situ study the EBV genomes were present in histiocytes, but not in B-cells. We could not confirm by ultrastructural study whether the histiocytes produced the EBV genome. The distribution of the silver grains was not clear; some cells showed grain deposition in the cytoplasm, but not in the nuclei. The cells may thus not produce EBV genomes. EBV-DNA was detected in the early macrophage cell line, which was established from the children with maturation defects of haematopoiesis (Revoltella et al. 1989) and viral particles of human immunodeficient virus type-1 (HIV-1) could be detected in the intracytoplasmic vacuoles of the macrophages of the lymph nodes (Tenner-Racz et al. 1988). The patient had an immunodeficiency state of decreased function and/or numbers of suppressor T-cells, which are usually increased in EBV infection (Frizzera 1987). The EBV genome could not be contained, and may have infected the histiocytes.

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