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CASE REPORT

Vanishing Bile Duct Syndrome Associated with Chronic EBV Infection

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KEY WORDS: bile duct loss; Epstein-Barr virus; *in situ* hybridization; HLA class II molecule.

Patients with infectious mononucleosis, the initial Epstein-Barr virus (EBV) infection, usually exhibit fever, pharyngitis, lymphadenopathy, and self-limited acute hepatitis, but rarely fulminant hepatitis or severe hepatitis occurs. Laboratory data show a mild and transient elevation of the aminotransferase level. We encountered a case of vanishing bile duct syndrome (VBDS) (1–4) due to chronic EBV infection. The patient demonstrated severe jaundice for one year, and this was complicated by virus-associated hemophagocytic syndrome and multiple small bowel perforations. Surgery was successfully performed. We examined serum EBV-DNA by polymerase chain reaction (PCR) and EBV mRNA in the liver by *in situ* hybridization. VBDS was first described by Neuberger et al (5) and was established by Sherlock in 1985 (2). She classified this syndrome into the following groups according to origin: developmental, immunological, infective, vascular, or chemical. Cytomegalovirus (CMV) is the most common virus that causes VBDS. However, our case indicated that chronic EBV infection can result in VBDS.

CASE REPORT

A 22-year-old male with no particular illness or relevant past medical history complained of fever, sore throat, and general malaise from the end of January 1994. He was admitted to a nearby hospital because jaundice developed. The first laboratory examinations showed total bilirubin 11.7 mg/dl, AST 326 IU/liter, ALT 334 IU/liter, LDH 1295 IU/liter, and alkaline phosphatase (ALP) 1496 IU/liter. Since intravenous glycyrrhizin was not effective, prednisolone (PSL) therapy was performed from February 22 to March 12. The total dosage, including the pulse therapy, was 15.7 g of prednisolone. However, the jaundice and liver dysfunction did not improve. Therefore, he was transferred to our hospital on March 15. On admission, he was conscious, body temperature was 37.8°C, and marked jaundice and splenomegaly were noted.

Laboratory Findings on Admission. Laboratory findings revealed a hemoglobin level of 11.5 g/dl; white cell count 4400/μl (neutrophils 92%); platelet count $8.5 \times 10^4/\mu$l; total bilirubin 29.7 mg/dl; direct bilirubin 18.7 mg/dl; AST 259 IU/liter; ALT 870 IU/liter; LDH 563 IU/liter; γ-GTP 745 IU/liter; ALP 2077 IU/liter; γ-globulin 0.42 g; and IgG 574 mg/dl, indicating the effects of a massive dose of PSL. Anti-nuclear antibody was negative, and anti-mitochondrial antibody was also negative, including immunoblotting. Hepatitis A, B, C, and G viral markers measured by commercially available kits were all negative. Complement fixation (CF) antibody-titer of CMV was 1:8, and CMV-DNA was negative. With regard to EBV infection, anti-viral capsid antigen (VCA) IgG was 1:160, anti-VCA-IgM was negative, both anti-early antigen (EA) IgG and anti-Epstein-Barr nuclear antigen (EBNA) were 1:10, and EBV-DNA (see below the methods) (6, 7) was positive. Anti-human immunodeficiency virus antibody was negative. Abdominal computed tomography showed lymph node swelling of the hepatic portal area and splenomegaly.

Clinical Course (Figure 1). After admission to our hospital, agranulocytosis was detected. Based on the findings of the bone marrow biopsy specimen, we diagnosed his blood disorder as virus-associated hemophagocytic syndrome (8). Moreover, he complained of abdominal pain and melena due to perforation of multiple intestinal ulcers. Therefore, subtotal intestinal resection and end-to-end anastomoses were performed. Sepsis, including bacterial peritonitis after the operation, persisted, and severe jaundice continued for about 1 year; however, he recovered and was discharged in April 1995. High titers of anti-VCA IgG and the EBV-DNA positivity continued. All liver function tests including ALP returned to normal by August 1996. At present, the patient exhibits no symptoms.

Liver Histology. The first liver biopsy specimen obtained in April 1994 showed severe frame disorganization in the parenchyma and bile duct loss (Figure 2a) and bile duct

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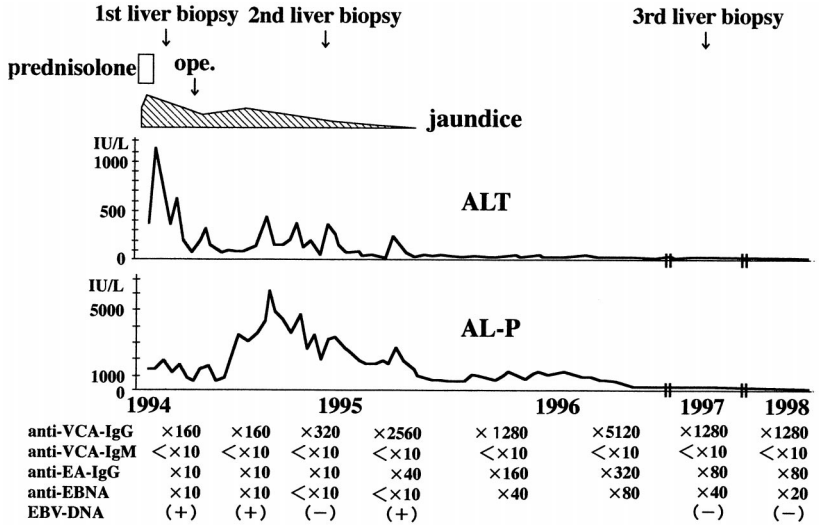

Fig 1. Clinical course and laboratory data of the patient. Alanine aminotransferase (ALT), alkaline-phosphatase (ALP), and various EBV markers are shown. Abbreviations: anti-viral capsid antigen (VCA); anti-early antigen (EA); and anti-Epstein-Barr nuclear antigen (EBNA).

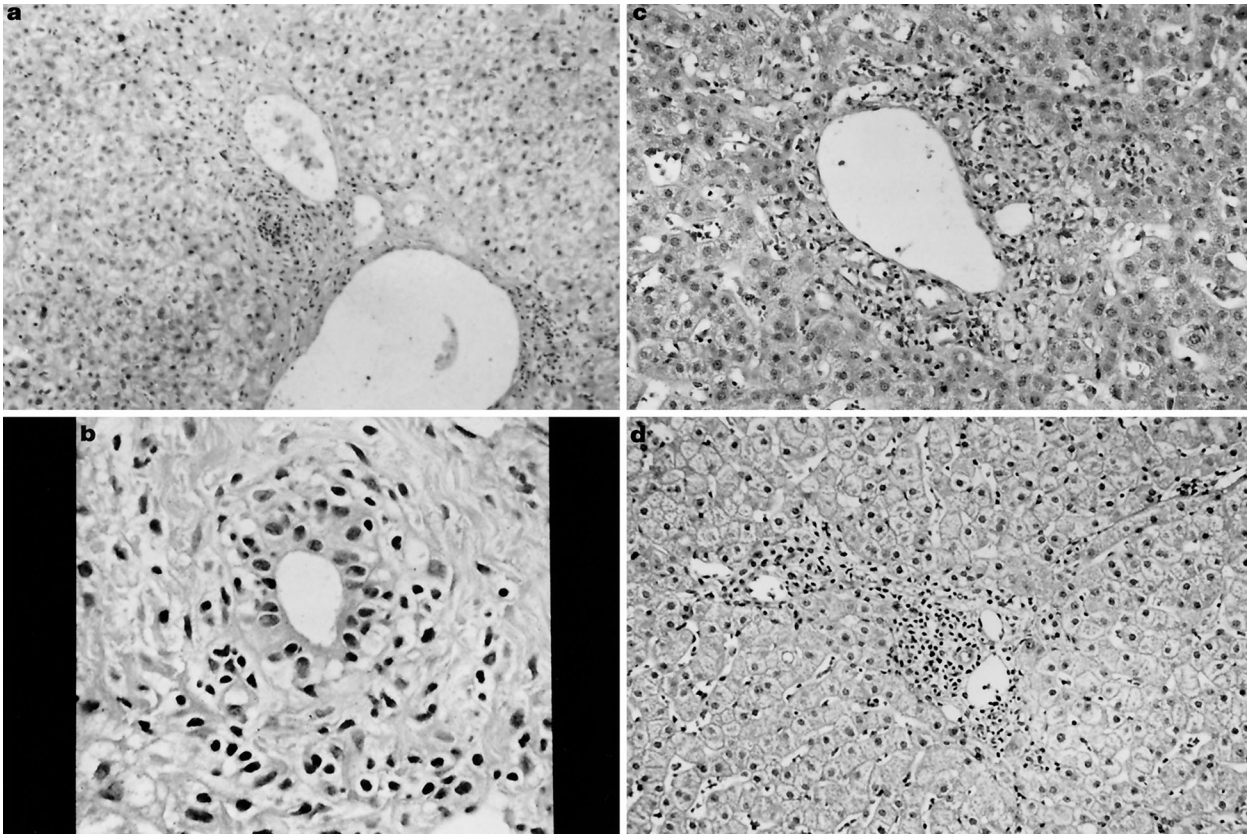

Fig 2. Liver biopsy findings (H&E stain, ×100). The first liver biopsy specimen taken in April 1994 shows severe frame disorganization in the parenchyma and bile duct loss (a) and bile duct destruction (b) in the portal area. However, no necroinflammation in either area is shown. The second liver biopsy specimen taken in November 1994 shows severe necroinflammation in both parenchyma and portal areas and bile duct loss (c). The third liver biopsy specimen taken in August 1997 shows marked improvement of necroinflammation and frame disorganization, but bile duct loss continues (d).

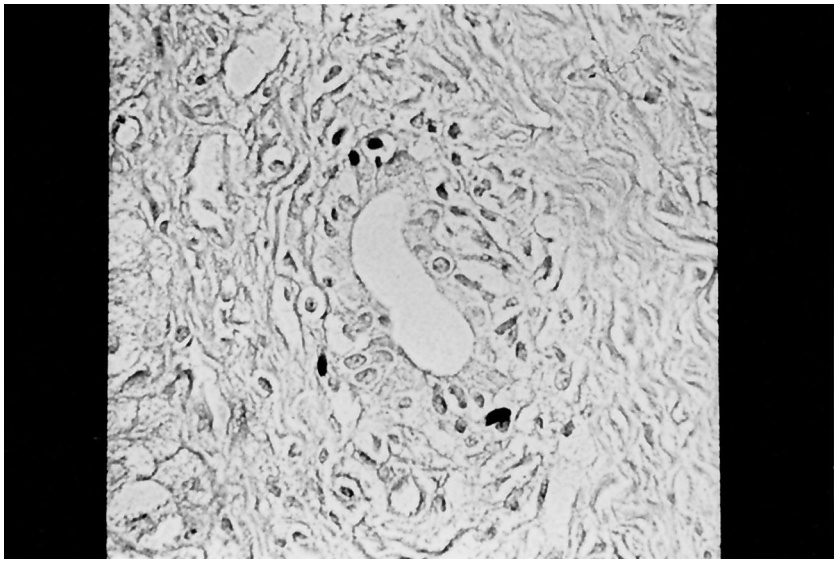

Fig 3. *In situ* hybridization. As a probe, ALP-linked oligonucleotide probe EBER-1 (EBV encoded small RNA, approximately 40 bp), which encoded the U1 site of the EBV genome (BamHI C site) was used and immunoscreening was performed on APS-coated paraffin-embedded first liver tissues. NBT-BCIP was used as dyeing substrate. The positive signal was detected on lymphocytes around the destructed bile ducts of the first biopsied liver tissue.

destruction (Figure 2b) in portal areas. However, no necroinflammation in either area was shown, perhaps due to the massive dose of PSL. The second liver biopsy specimen obtained in November 1994 showed severe necroinflammation in both parenchyma and portal areas and bile duct loss (Figure 2c). The third liver biopsy specimen obtained in August 1997 showed marked improvement of necroinflammation and frame disorganization but bile duct loss remained (Figure 2d).

Serum EBV-DNA by PCR. As a specific primer, 161 bp of the BamHI W site, which encoded the IR1 site of the EBV genome, was synthesized based on the published DNA sequences of Baer et al (9). Serum EBV-DNA was detected from March 1994 to February 1995.

***In Situ* Hybridization.** To confirm the presence of EBV mRNA in the liver tissue, *in situ* hybridization was performed (10–12). As a probe, an ALP-linked oligonucleotide probe, EBER-1 (EBV encoded small RNA, approximately 40 bp), which encoded the U1 site of the EBV genome (BamHI C site) was used and immunoscreening was performed on 3-aminopropyltriethoxysilane (APS) -coated paraffin-embedded liver tissue. Finally, the stains were visualized with 5-bromo-4-chloro-3-indoxylphosphate/nitroblue tetrazolium chloride (BCIP/NBT) substrate.

A positive signal was detected on lymphocytes around the destructed bile ducts of the first biopsied liver tissue (Figure 3). No positive signals were detected in the second or third biopsied liver tissue.

EBV-DNA in Liver Tissue by PCR. The homogenized third biopsied liver sample was examined by PCR (6, 7). The PCR results are shown in Figure 4. Lane 2 corresponds to the PCR product revealing the patient's liver tissue, lane 3 corresponds to the internal control, lane 4 corresponds to the negative control, and lanes 5–8 correspond to the PCR products of the positive control. As shown in lane 2, a positive PCR result was obtained, indicating that EBV replicated in the liver tissue at this time.

Immunohistochemical Study. Anti-HLA class II (DP, DQ, DR β chain) (M0775, Dako, Santa Barbara, California) was immunohistochemically stained by the labeled streptavidin–biotin method using ethanol-treated formalin-fixed, paraffin-embedded liver tissue (13). Biotin-labeled anti-human immunoglobulin as a second antibody and peroxidase conjugated streptavidin as a third antibody were used. Finally, the stains were visualized with diaminobenzidine substrate. Anti-HLA class II molecule was diffusely

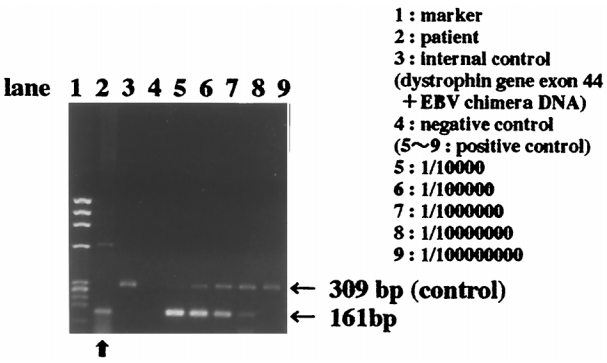

Fig 4. EBV-DNA in liver tissue by polymerase chain reaction. Homogenized third biopsied liver sample is shown. PCR was performed for the examination. Lane 2 corresponds to the PCR product revealing the patient's liver tissue, lane 3 corresponds to the internal control, lane 4 corresponds to the negative control, and lanes 5–8 correspond to the PCR products of the positive control. As shown in lane 2, a positive PCR result was obtained.

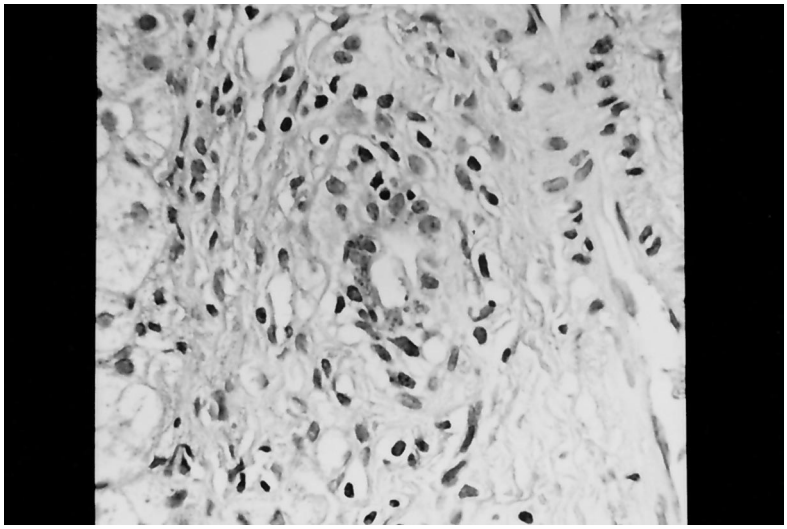

Fig 5. Immunohistochemistry of anti-HLA class II. Anti-HLA class II (DP, DQ, DR β chain) (M0775, DAKO) was stained by labeled streptavidin biotin method using ethanol treated formalin-fixed, paraffin-embedded first biopsied liver tissue. Biotin-labeled anti-human immunoglobulin as a second antibody and peroxidase conjugated streptavidin as a third antibody were used. Anti-HLA class II is diffusely stained on the bile duct epithelium of the first biopsied liver specimen.

stained on the destructed bile duct epithelium of the first biopsied liver specimen (Figure 5).

DISCUSSION

Our patient was diagnosed as having EBV infection because serum EBV-DNA was detected by PCR and an mRNA positive result for EBV was found on the biopsied liver specimen by *in situ* hybridization. Moreover, since EBV-DNA was detected in the third biopsied liver specimen, chronic EBV infection had developed for at least three years. EBV infection appears to have a self-limited course. However, EBV infection may occasionally induce fulminant hepatitis (14). Our patient may have been induced by a massive dose of PSL. It is well-known that PSL, a strong immunosuppressive agent, stimulates viral replication.

VBDS was first designated as bile duct loss due to chronic graft-versus-host disease (GVHD) after orthotopic liver transplantation (5). Sherlock classified this syndrome into five groups according to the origin—developmental, immunological, infective, vascular, or chemical (2). Some investigators have referred to these symptoms with an unknown origin as idiopathic adulthood ductopenia (3).

Among the infective origins, CMV is the most common agent that causes VBDS (15–17). However, in most cases of CMV infection, congenital or secondary host immunodeficiency observed in patients with AIDS (16, 17) or those receiving liver transplantation (1) are also detected. To our knowledge, no study has previously reported VBDS associated with chronic EBV infection. EBV infection indicating lymphoproliferative disorder resembling acute cellular rejection has been reported (18–20). However, in this case, massive doses of PSL indicated host immunodeficiency as shown in CMV infection associated with VBDS.

Regarding severe complications, our patient exhibited virus-associated hemophagocytic syndrome and small bowel perforations. As previously reported (21–24), EBV infection sometimes causes fatal conditions. However, our patient survived.

The immunological mechanism of VBDS remains controversial. Recent immunohistochemical studies indicated aberrant HLA class II molecules expressed on the bile duct epithelium of cases of GVHD (25–27), primary biliary cirrhosis (28–30), or CMV infection (31–33), all of which may lead to VBDS (2). Our immunohistochemical studies showed aberrant HLA class II molecules expressed on the bile duct epithelium of this patient. Therefore, aberrant HLA class II molecules may play an important role in the immunological development of VBDS.

SUMMARY

We reported here an adult patient with vanishing bile duct syndrome due to chronic EBV infection. A 22-year-old male was admitted to a nearby hospital complaining of a sore throat and jaundice. He received a high dose of prednisolone for bile stasis of acute viral hepatitis. However, the hepatitis did not improve, and he was transferred to our hospital. He had exhibited jaundice for one year as well as hemophagocytic syndrome and intestinal perforation. Subtotal intestinal resection was successfully performed. Three follow-up biopsied liver specimens indicated vanishing bile duct syndrome. Positive results of EBV-DNA in his serum and mRNA of EBV by *in situ* hybridization of his liver indicated that massive doses of prednisolone caused chronic EBV infection and vanishing bile duct syndrome.

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