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Case report

Epstein-Barr virus infection resembling autoimmune hepatitis with lactate dehydrogenase and alkaline phosphatase anomaly

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Abstract: A 73-year-old man had fever, lymphadenopathy, granulocytopenia, thrombocytopenia, ascites, pleural effusion, liver injury, and an allergic-like skin rash. Autoantibodies, such as anti-nuclear antibody, were shown, and there were lactate dehydrogenase and alkaline phosphatase anomalies and platelet-associated IgG. His liver injury resembled that in autoimmune hepatitis. He was diagnosed with Epstein-Barr virus (EBV) infection associated with autoimmunization because of his clinical course, fluctuation of anti EBV antibodies and positive EBV genome in circulating lymphocytes and serum. This case suggests a close relationship between EBV infection and autoimmunization or autoimmune-like hepatitis.

Key words: Epstein-Barr virus infection, autoimmune hepatitis, autoimmunization, lactate dehydrogenase anomaly, alkaline phosphatase anomaly

Introduction

Epstein-Barr virus (EBV) infection is associated with a broad spectrum of clinical manifestations, depending on the immune status of the host. Recently two kinds of clinical entities due to EBV infection have been elucidated: infectious mononucleosis due to primary EBV infection, and chronic active EBV infection (CAEBV). Chronic active Epstein-Barr virus infection, initially reported by Tobi et al.,[1] has received marked attention because of its various clinical manifestations and grave prognosis. It has been postulated that symptoms of CAEBV result from the reactivation of persistent and silent EBV infection or from lasting activity following primary EBV infection. Fever, lymphadenopathy, hepatosplenomegaly, hematological cytopenia, and skin eruptions are the main signs and symptoms of CAEBV.[2,3] CAEBV is frequently associated with lymphoproliferative diseases, including virus-associated hemophagocytic syndrome, granular lymphocyte proliferative disorder, and malignant lymphoma;[4,5] however, the pathogenetic details remain unknown. We report a case of EBV infection in a patient who had various immune abnormalities and autoimmune-like hepatitis.

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Case report

A 73-year-old Japanese man developed fever (temperature more than 38°C) in February 1997. He consulted a local hospital and was treated with cefpodoxime proxetil. Two weeks later he developed appetite loss and abdominal distention, with prolonged fever, and he was referred to our hospital on March 7, 1997.

None of his brothers or children had had autoimmune or cryptogenic hepatitis. He had had no history of persistent fever, lymphadenopathy, or hepatitis, except for elevated lactate dehydrogenase (LDH) level shown on a physical checkup a year before admission. On admission, his temperature was 37.5°C. Superficial lymph nodes were not palpable, and a skin rash was observed on the right side of the abdomen. Abdominal lymphadenopathy (Fig. 1a), ascites, and right pleural effusion were detected by computed tomography. Laboratory data on admission are shown in Table 1. Granulocytopenia, thrombocytopenia, and eosinophilia were present in the peripheral blood. Biochemical data indicated liver injury, with conspicuously elevated LDH and alkaline phosphatase (ALP) levels. Both LDH and ALP demonstrated abnormal isozyme patterns (i.e., anomalies). These LDH and ALP anomalies were shown to be complexes containing each enzyme and the corresponding specific IgG by an immunofixation technique following electrophoresis (Fig. 2). Anti-nuclear

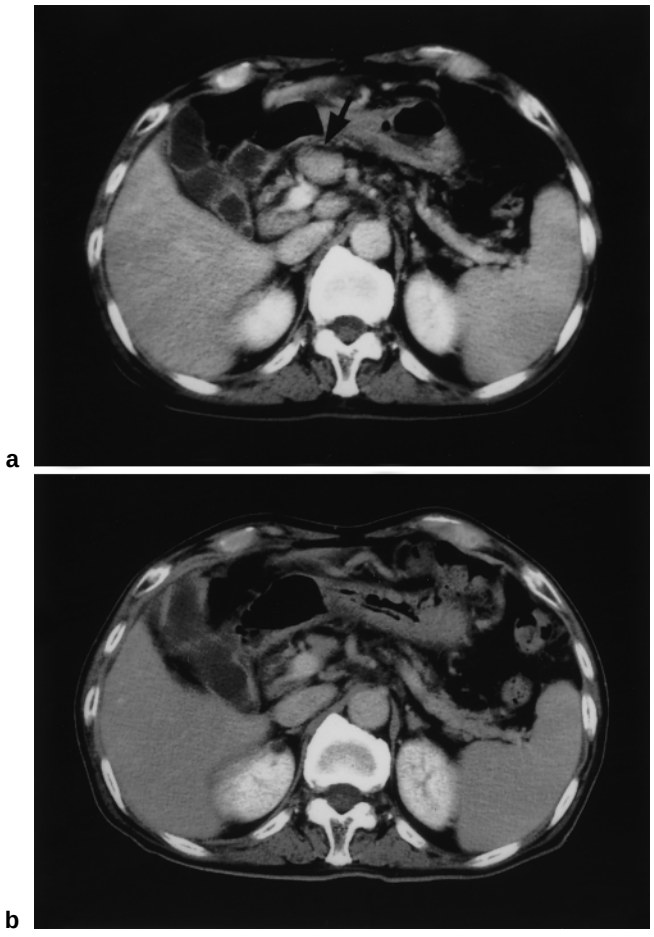

Fig. 1a,b. Computed tomography scans. **a** On admission, shows abdominal lymphadenopathy (*arrow*). **b** Five weeks after admission, lymphadenopathy had disappeared

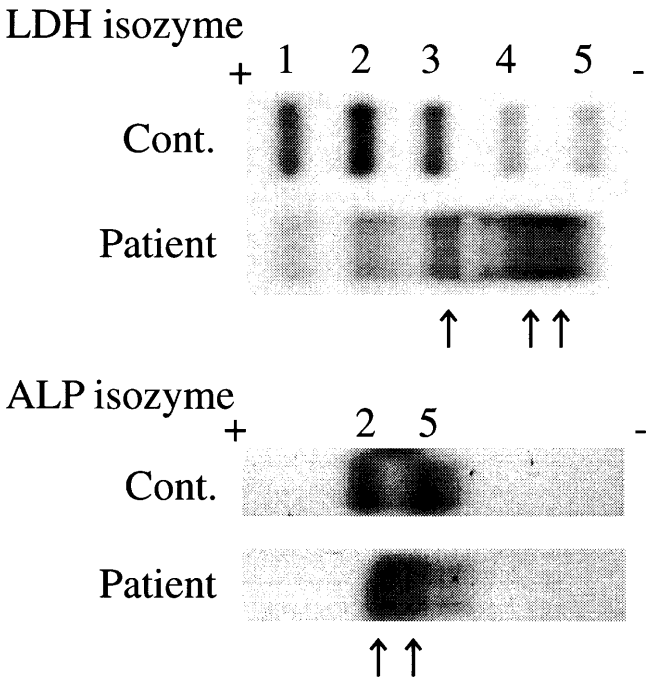

Fig. 2. Electrophoresis patterns of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) isozymes. *Arrows* show abnormal bands. *Cont.*, Control

Table 1. Laboratory data on admission

RBC	443 × 10^4/μl	TP	9.3 g/dl	ANA	300 ×
Hb	14.2 g/dl	Alb	2.5 g/dl	Anti-dsDNA Ab	21.2 IU/ml
Ht	42.5%	T-bil	2.8 mg/dl	Anti-ssDNA Ab	130.3 AU/ml
WBC	5800/μl	D-bil	1.6 mg/dl	Anti-microsome Ab	(−)
Stab	7%	AST	62 IU	AMA	(−)
Seg	19%	ALT	35 IU	AMA-M2	(−)
Lym	15%	LDH	1667 IU	LE cell	(−)
Mon	10%	LDH1	6.0%	PA-IgG	69.3 ng/10^7cells
Eos	47%	LDH2	14.5%	CRP	2.8 mg/dl
Bas	2%	LDH3	19.5%*	IgG	4160 mg/dl
Plt	4.8 × 10^4/μl	LDH4	33.2%*	IgA	417 mg/dl
ESR	100 mm/h	LDH5	26.3%*	IgM	227 mg/dl
PT	72.0%	*anomaly (IgG)		IgE	652 IU/ml
APTT	38.6 s	ALP	1418 IU	HBs Ag	(−)
		ALP1	5.5%	HBs Ab	(−)
		ALP2	13.7%*	HCV Ab	(−)
		ALP3	80.8%*	HCV RNA	(−)
		*anomaly (IgG)		IgM-HA Ab	(−)
		γ GTP	222 U		

Underlines represent abnormal data. Normal ranges for these abnormal data were: Plt, 16–30 × 10^4/μl; ESR, <15 mm/h; Alb, 3.7–5.5 g/dl; T.bil, 0.1–1.2 mg/dl; D.bil, 0.1–0.4 mg/dl; AST, 11–32 IU; LDH, 140–360 IU; ALP, 66–220 IU; γ GTP, 8–33 U; ANA, <80; PA-IgG, <25 ng/10^7 cells; CRP, <0.3 mg/dl; IgG, 870–1700 mg/dl; IgE, <160 IU/ml

anti-VCA-IgG			640	640	640	640
anti-VCA-IgM			<10	<10	<10	<10
anti-EBNA			40	160	80	20
anti-EBEA-IgG			320	80	20	40
anti-EBEA-IgA			80			20
anti-EA-DR-IgG			<10			<10
IgG	4160	3420		3260		
ANA	320	160				160

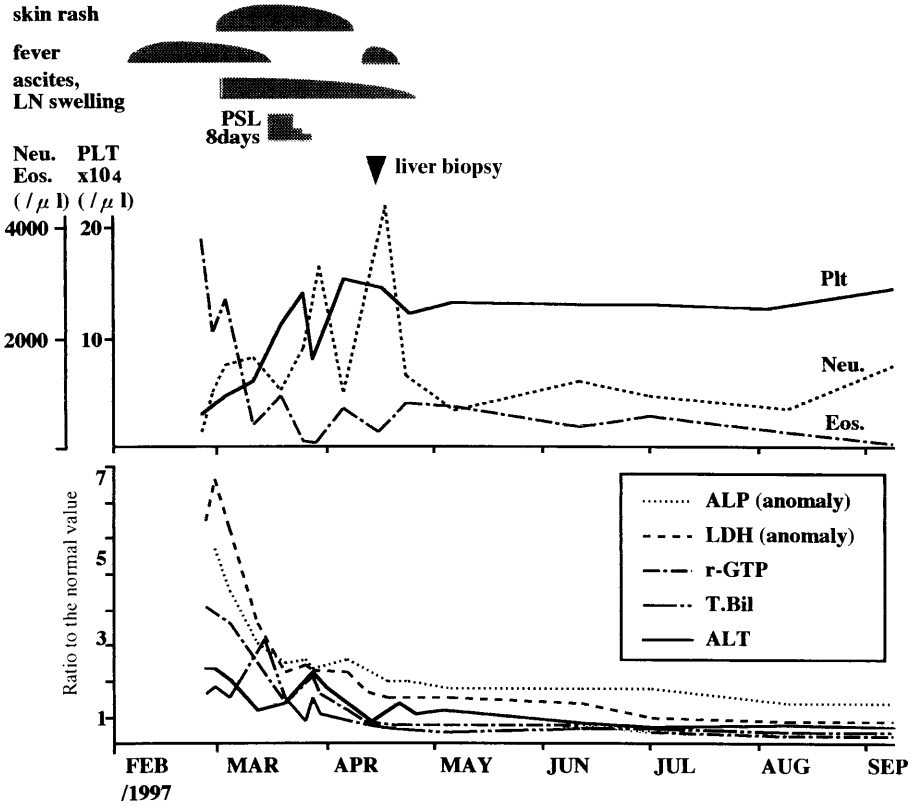

Fig. 3. The patient's clinical course. Numbers on the vertical axis of the *lower* panel denote ratios of ALP, LDH, *r*-glutamyl transpeptidase (*r-GTP*), total bilirubin (*T.bil*), and alanine aminotransferase (*ALT*) to the upper limit of normal values. *LN*, Lymph node; *PSL*, prednisolone; *PLT*, platelets. See text for antibody definitions

antibody (ANA) and platelet-associated IgG (PA-IgG) were positive. In bone marrow aspirates, findings were normal except for an increase in eosinophils.

The patient's clinical course is shown in Fig. 3. As we had initially assumed that he had developed cholangitis which followed malignancy of bile duct or pancreas, we administered antibiotic (ampicillin sodium/sulbactam sodium) and then replaced these by cefoperazone sodium/sulbactam sodium. Immediately after admission, the granulocytopenia, thrombocytopenia, and liver injury were alleviated to some extent. The skin rash noted on the right side of the abdomen on admission had spread to his back and face since antibiotic treatment. As drug allergy was suspected, an initial dose of 30 mg prednisolone per day was given, then tapered off for 10 days. The skin rash was alleviated with the prednisolone therapy. Lymphocyte stimulating tests against cefpodoxime proxetil, ampicillin sodium/sulbactam sodium, and cefoperazone sodium/sulbactam sodium were negative. Cytopenia and liver function test results, except for LDH and ALP bound with specific IgGs, were normalized within 5 weeks after admission. At the same time, the pleural effusion, ascites, and abdominal lymphadenopathy had disappeared (Fig. 1b). The diagnosis of malignant tumor was excluded because of the patient's clinical course.

To investigate the cause of the liver injury, a liver biopsy was performed (Figs. 4, 5). Histologically, fibrous portal zones had expanded with moderate inflammatory infiltration, with mild piecemeal necrotic foci. Hepatocyte changes, such as multinuclearizaton, anisocytosis, and scattered areas of small focal necrosis were seen. There was a portal-portal bridging formation in the entire sample. Histological findings revealed the regeneration of hepatocytes caused by severe hepatocellular necrosis, and the findings were compatible with

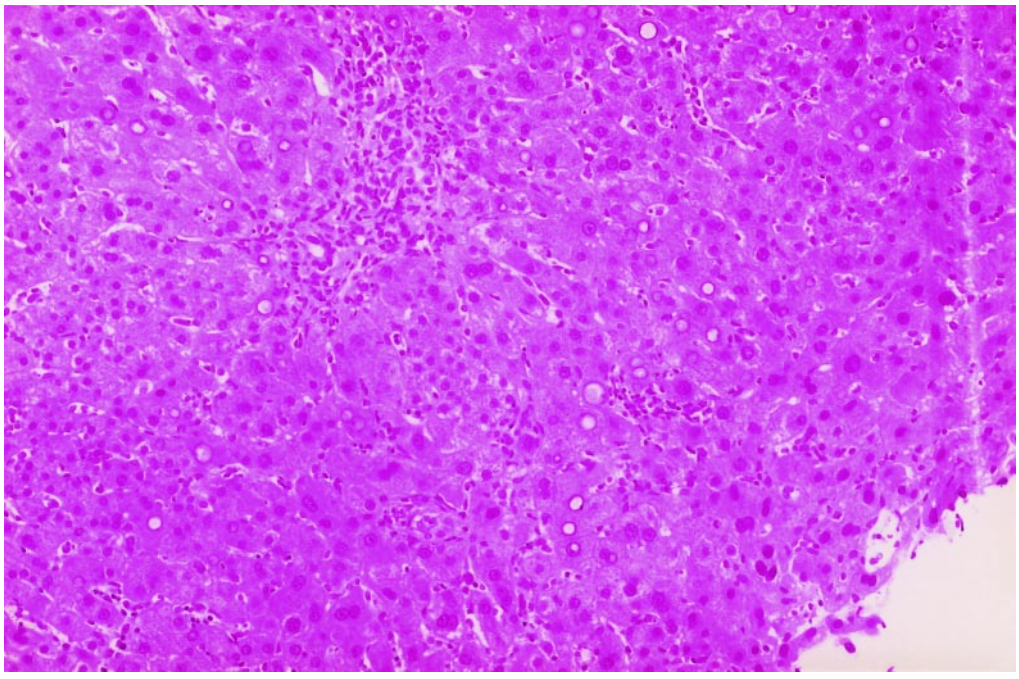

Fig. 4. Histological findings of the liver. Mild inflammatory infiltration, multinuclearization, and anisocytosis were conspicuous. Mild focal necrosis was observed. H&E, × 100

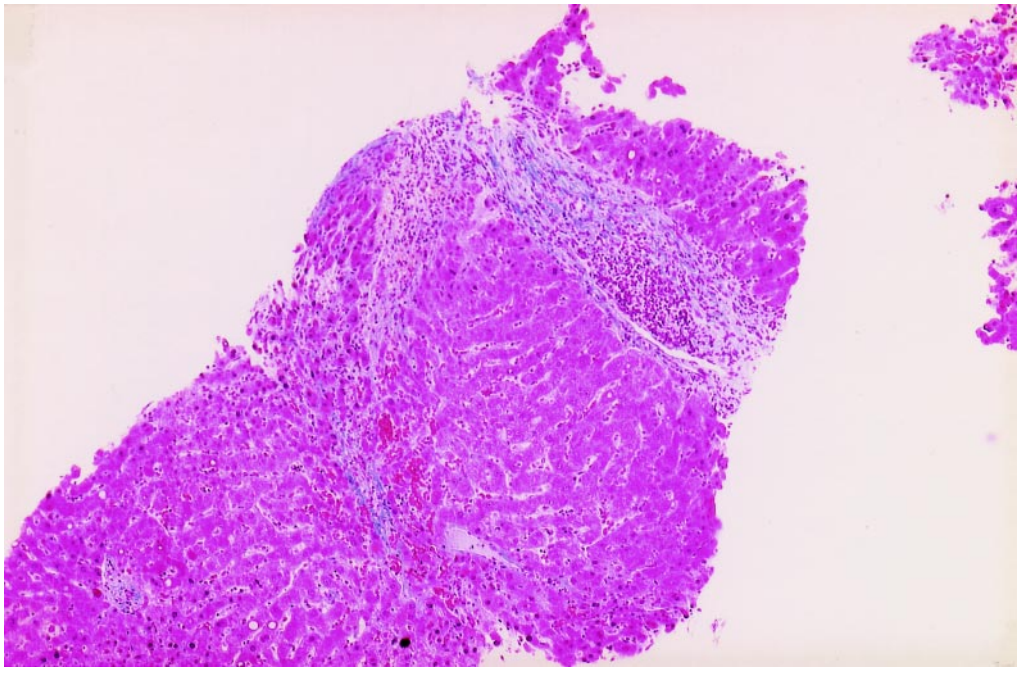

Fig. 5. There was a portal-portal bridging formation in the entire sample. In this area, moderate portal inflammatory cell infiltration with moderate piecemeal necrosis was observed. Azan, × 40

the pathological features of chronic hepatitis (New Inuyama histological classification: F_{1-2}, A_1).[6]

Serological EBV markers were measured in May for the first time. Anti-EBV capsid antigen-IgG antibody (VCA-IgG) and anti-early-antigen-IgG antibody (EBEA) were markedly elevated and anti-EBV nuclear antigen antibody (EBNA) was positive. EBV genome was detected in 2 of 50000 circulating lymphocytes by in situ hybridization with an origonucleotide probe deduced from the coding sequence of EBV-encoded small RNA-1 (EBER-1)[7] (Fig. 6). In hepatocytes, the EBV genome was not detected by in situ hybridization with the same probe. EBV DNA was detected in serum and peripheral blood mononuclear cells by polymerase

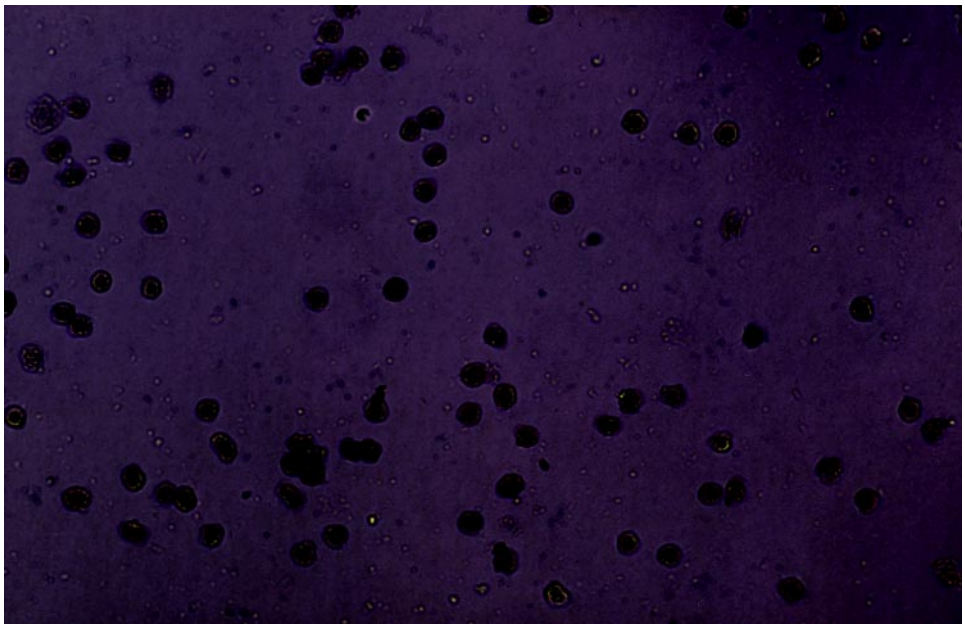

Fig. 6. In situ hybridization of circulating lymphocytes. An Epstein-Barr virus-encoded small RNA-1 (EBER-1) RNA-positive lymphocyte, stained dark, can be seen. Two of 50000 cells were positive for EBER-1 RNA

Table 2. Detection of Epstein-Barr virus (EBV) by molecular biologic techniques

Tissue (date)	Method	Probe/Primer	Result
Liver (1997.4.16)	In situ hybridization	EBER	(−)
Circulating lymphocytes (1997.5.2)	In situ hybridization	EBER	(+) 2/5 × 10^4 cells
Peripheral blood (1997.5.2)	PCR	IR1	(+)
Serum (1997.5.2)	PCR	IR1	(+)

Probe for in situ hybridization, deduced from the coding sequences of the EBER-1 (EBV-encoded small RNA-1) region
Primers for polymerase chain reaction (PCR), deduced from the coding sequences of the *Bam*HI-W (IR1) region

chain reaction (PCR) (Table 2). The PCR assay of EBV DNA was done according to a previous method,[8] with some modification, using the original primer set, sense primer 5′ TCC TCG TCC AGC AAG AAG AG 3′ and anti-sense primer 5′ CAA CTT GAG GCA GCC TAA TCC 3′, deduced from DNA sequences within EBV's *Bam*HI-W (internal repeat 1; IR1) region. The titer of EBEA gradually dropped with the improvement of clinical signs and alleviation of symptoms. The findings of the EBV markers suggested that the patient's symptoms had been associated with EBV infection. After discharge in June, his cytological and biochemical data were almost normalized.

Discussion

Primary EBV infection-related infectious mononucleosis is known to be a cause of immune abnormalities and lymphoproliferative disorders. Further, various manifestations caused by persistent or recurrent EBV infection have been reported since the initial report by Tobi et al.[1] of CAEBV. EBV has evolved a number of strategies to evade the immune system, including latency with restricted gene expression, interference with cytokines, and interference with cytotoxic T cells.[9] CAEBV is considered to occur when these systems are inoperative. CAEBV patients show both humoral and cellular immune abnormalities and have infectious mononucleosis-like symptoms, hematological cytopenia, chronic hepatitis, interstitial pneumonia, and uveitis.[2,3] Lymphoproliferative disorders are also associated.[4,5] Diagnosis of CAEBV is made from clinical symptoms, unusual pattern of anti-EBV antibodies, and by detection of the EBV genome. The criteria were demonstrated by Rickinson[2] and Straus.[3]

Our patient had fever, abdominal lymph node enlargement, ascites, pleural effusion, skin rash with eosinophilia, thrombocytopenia, neutropenia, and pathological chronic hepatitis. A number of autoanti-

bodies, such as ANA and PA-IgG, and anomalies of LDH and ALP were also detected. There was no doubt, from the fluctuation of EBV-related antibodies and EBV proliferation confirmed by molecular biologic techniques,[4,10] that the serial symptoms resulted from EBV infection. His advanced age, of 73, and lack of increase in atypical lymphocytes were not typical of primary EBV infection.[11] A diagnosis of CAEBV was suspected, because of his history of abnormal LDH noted a year prior to admission, along with the above-mentioned findings. The titer of VCA-IgG was remarkably high for his age but the pattern of EBV antibodies seemed to be indistinguishable from that of primary infection. Portal-portal bridging consisted of loose fibrosis, so pathological findings of the liver did not indicate chronic or intermittent hepatitis. There was no associated lymphoproliferative disorder shown by hematological examination and bone marrow findings. However, because prednisolone was administered, such a complication cannot be completely ruled out.

Pullen et al.[12] reported that infectious mononucleosis tended to complicate skin eruptions due to drug allergy. The relationship between infectious mononucleosis and drug allergy has been discussed. Olson et al.[13] reported that allergic patients with CAEBV had significantly increased responsiveness to specific allergen, and increased numbers of IgE-positive lymphocytes and elevated serum levels of IgE compared with those who had allergies in the absence of CAEBV. It was also reported that a significant increase in EBV nuclear antigen-positive cells occurred only in B cells obtained from patients with CAEBV when cells were stimulated with specific antigens.[14] In our patient, the skin rash was a typical hypersensitivity rash, as indicated by the eosinophilia, increased IgE level, and the favorable effect of prednisolone. EBV carriers with activated EBV, such as those with CAEBV or infectious mononucleosis, may be more sensitive to some drugs than those with latent EBV infection, as demonstrated by Nazareth.[15]

Many autoantibodies were detected in our patient, as follows: ANA, anti-DNA antibody, PA-IgG, and IgG bound to LDH and ALP. Initially the diagnostic process was confused because the LDH and ALP anomalies modified the clinical picture of liver injury. It is assumed that immunoglobulin-bound enzymes induce autoantibodies, and these are likely to be detected in patients with chronic liver disease or ulcerative colitis. Yamamoto and Hattori[16] showed that the incidence of double anomalies of LDH and ALP in Japanese was 0.04%, and the appearance of these anomalies was associated with some underlying diseases. LDH and ALP anomalies are considered to be an outcome of immune abnormalities. These anomalies, together with other autoantibodies in our patient, appeared to result from CAEBV. There are many reports concerning the high incidence of EBV infection associated with systemic lupus erythematosus,[17] Sjogren's syndrome,[18] and other autoimmune diseases. It has been suggested that autoimmune diseases occur in a setting of cross-reactive antibodies, based on the mimicry of epitopes between host proteins and the EBV proteins.[19,20] However, the occurrence of autoantibodies related to EBV infection cannot be explained entirely by antibodies to mimicking epitopes. Previous explanations of the occurrence of autoantibodies during EBV infection are as follows: polyclonal B cell stimulation by infecting EBV and the consequent production of polyreactive autoantibodies from B cells, virus-specific host cell damage and resultant autoimmunization by the released autoantigenic products, and the formation of a specific viral/host complex with a consequent adjuvant effect favoring autoantibody production.

Generally, none or a small number of EBV-positive hepatocytes were observed in patients with EBV-related liver injury.[21,22] The mechanism of liver injury associated with EBV infection is not yet apparent. Vento et al.[23] have recently reported two patients with autoimmune hepatitis caused by EBV infection. In their patients, there was a defect in suppressor-inducer T lymphocytes specifically controlling the immune response to the asialoglycoprotein receptor. The antibodies to this autoantigen persisted and increased after infectious mononucleosis, and autoimmune hepatitis developed. It is suggested that autoantibody to asialoglycoprotein receptor, which is expressed on the hepatocellular membrane as a liver-specific antigen, contributes to the pathogenesis of autoimmune hepatitis.[24] In our patient, EBV was not found in hepatocytes and the pathological evidence resembled that of autoimmune hepatitis. Laboratory findings in our patient seemed to support the diagnosis of autoimmune hepatitis. Our patient was diagnosed with probable autoimmune hepatitis by the scoring system of the International Hepatitis Group. It is interesting to note that an autoimmune mechanism may contribute to EBV-related liver injury.

Thrombocytoenia in our patient appearred to be due to autoantibody-mediated platelet destruction, because PA-IgG was positive and the number of megakaryocytes in bone marrow was not decreased. We believe that PA-IgG, platelet autoantibody, occurred as a result of the reactivation of EBV. Patients with primary EBV infection with thrombocytopenia were reported by Pipp et al.[25] Regarding granulocytopenia, we did not measure anti-granulocyte antibody. But it could not be denied that our patient's granulocytopenia also originated from autoantibody-mediated granulocyte destruction or hypoproduction during EBV infection, as demonstrated by Schooley et al.[26]

Investigations to elucidate the differences between CAEBV and infectious mononucleosis and the mechanisms of reactivation of EBV as CAEBV have only just began. Further work on the autoimmunization, hypersensitivity, and liver injury associated with EBV infection needs to be done. The patient we have described was characterized by EBV-related autoimmunization. When patients with complex immune abnormalities of unknown origin are investigated, EBV infection should be considered as one of the differential diagnoses.

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