

Clonal T-cell Lymphoproliferation Containing Epstein-Barr (EB) Virus DNA in a Patient with Chronic Active EB Virus Infection

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A 10-year-old boy with chronic active Epstein-Barr virus (EBV) infection developed T-cell lymphoproliferation in the terminal stage of hepatic failure. The phenotypes of the proliferating lymphocytes were CD3⁺, CD4⁺ and HLA-DR⁺. The genomic DNAs from these cells demonstrated two rearranged T-cell receptor β -chain genes and contained the EBV genome. These findings indicate that EBV can infect T lymphocytes and might cause clonal T-cell lymphoproliferation.

Key words: Epstein-Barr virus — Chronic EB virus infection — Clonal lymphoproliferation — T lymphocyte

Epstein-Barr virus (EBV) has been implicated as the causative agent in infectious mononucleosis and has been shown to be associated with Burkitt's lymphoma and nasopharyngeal carcinoma.¹⁾ Recently it appears that EBV is also associated with lymphoproliferative disorder in immunodeficiency,²⁾ thymic lymphoepithelial carcinoma³⁾ and Sjogren's syndrome.⁴⁾ The EBV genome has been found only in B lymphocytes, oropharyngeal epithelial cells, thymic lymphoepithelial carcinoma cells and salivary gland duct cells.

Recently T-cell lymphomas following chronic active EBV infection were reported,⁵⁾ which suggested that EBV involves not only B lymphocytes but also T lymphocytes. Similarly, we experienced a patient who had suffered from chronic active EBV infection for eight years and thereafter developed clonal T-cell lymphoproliferation.

The patient did not have any symptoms of immunodeficiency until he developed a fever at the age of 2 years and 5 months in April 1978. Although hepatosplenomegaly was pointed out at that time, no diagnostic examinations were carried out. Thereafter periodic manifestations of fever and hepatosplenomegaly continued for 3 years. In September 1981, he was admitted to a local hospital due to a fever, anemia and liver dysfunction. For the first time, EBV infection was diagnosed on the basis of the clinical symptoms and antibody titers to EBV. Although he was treated with high-dose gammaglobulin, acyclovir and prednisolone, the symptoms persisted and the antibody titers to EBV were of an abnormal pattern consistent with chronic active EBV infection (Table I). The patient was referred to us in June 1986 for treatment with interferon- γ and/or interleukin-2.⁶⁾ On admission, however, the liver dysfunction

was critical and leukocytosis in the blood was demonstrated. The increased lymphocytes were activated and expressed CD3, CD4 and HLA-DR antigens, as previously reported⁷⁾ (Table II). At that time, antibodies to human T-lymphotropic virus (HTLV-1) were not detected. His liver dysfunction was progressive, and thereafter coagulopathy and disturbance of consciousness developed. Twenty-six days after admission, the patient died of liver failure. No autopsy was performed.

To investigate for the presence of EBV DNA and clonality of the proliferating lymphocytes, we carried out Southern blotting as reported previously.⁸⁾ DNA was extracted from the peripheral blood mononuclear cells obtained on June 26 and stored at -80°C . The DNA (5 μg) was digested with 50 units of restriction endonuclease *Bam*HI or *Eco*RI at 37°C for 3 h. The digested DNA was subjected to electrophoresis in a 0.8% agarose gel and transferred to a nylon filter by the

Table I. Antibody Titers to EBV

Date	VCA IgG	VCA IgM	EA-DR IgG	EBNA
81/10/27	1280	80	1280	640
82/5/11	2560	80	1280	640
9/2	2560	640	2560	640
84/8/31	5120	<10	640	320
85/11/28	640	<10	640	160
86/5/15	10240	<10	1280	640
6/19	2560	<10	640	80
7/2	1280	<10	80	80

VCA, viral capsid antigen; EA-DR, early antigen diffuse and/or restricted component; EBNA, Epstein-Barr nuclear antigen.

Table II. WBC Differential and Phenotype of Lymphocytes

	Date	'86 5.19	6.19	6.26	7.7	7.12
WBC	(/μl)	4,600	2,400	6,100	2,890	19,200
Neutrophils	(%)	41	29	1	44	6
Eosinophils	(%)	0	0	0	0	0
Basophils	(%)	1	4	0	0	0
Lymphocytes	(%)	57	64	98	54	86
Monocytes	(%)	1	2	1	2	0
Atypical lymphocytes	(%)	0	1	0	0	8
CD3(Leu4)	(%)	93.4			98.3	
CD4(Leu3)	(%)	83.1			90.1	
CD8(Leu2)	(%)	22.5			11.0	
CD20(B1)	(%)	1.3			1.0	
CD4 ⁺ HLA-DR ⁺	(%)	56.3			83.9	
CD8 ⁺ HLA-DR ⁺	(%)	11.7			2.3	

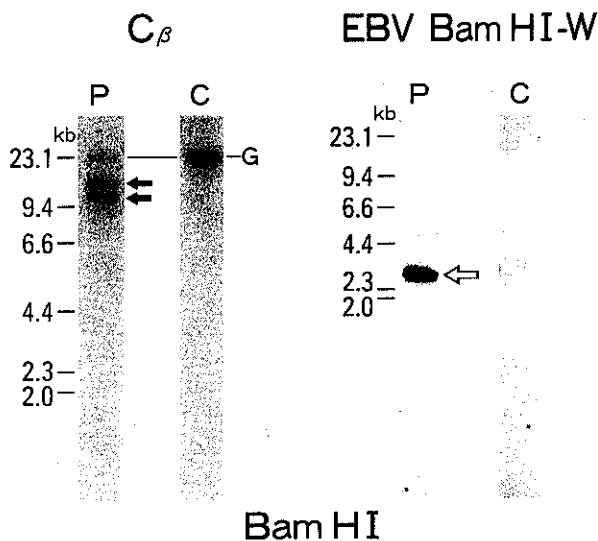


Fig. 1. DNA (5 μg in each lane) was digested with *Bam*HI. After hybridization with the T-cell receptor β-chain gene probe (*Cβ*1), two rearranged bands (closed arrows) were observed in lane P (DNA from peripheral mononuclear cells of the patient). Lane C (control DNA from EBV-negative Burkitt's lymphoma cell line, BL-42) demonstrated the germline configuration (G). After hybridization with the EBV *Bam*HI-W probe, a 3-kb band of EBV DNA (open arrow) was detected. The control revealed no fragment of EBV DNA.

Southern blotting technique.⁹⁾ Filter-bound DNA fragments were then hybridized to ³²P-labeled probes and visualized on an autoradiogram.

After digestion with *Bam*HI and hybridization with the EBV *Bam*HI-W probe,¹⁰⁾ a 3-kb band of EBV DNA

which corresponds to EBV *Bam*HI-W fragment was detected (Fig. 1). Similarly, when the probe containing the tandem terminal repeated sequences of the EBV genome¹¹⁾ was used, a single band representing a clonal proliferation of given cells was detected (data not shown).

While no rearranged bands were observed after digestion with *Eco*RI and hybridization with the immunoglobulin heavy chain *J_H* gene probe¹²⁾ (data not shown), two rearranged bands were demonstrated after digestion with *Bam*HI and hybridization with the T-cell receptor *Cβ*1 gene probe¹³⁾ (Fig. 1).

If contaminated B-cells had affected our present results, rearranged immunoglobulin *J_H* genes should have been detected; however, if present, they were below the detection limit of our technique. As was the case in the previous report,¹⁴⁾ we were not able to detect EBV genome in samples from patients with acute infectious mononucleosis in the same manner.

These findings suggest that the cells containing the EBV genome were of clonal origin and the clonally proliferating lymphocytes were of T-cell origin. Therefore, the EBV-infected cells are surmised to have been the clonally proliferating T lymphocytes.

More recently, Jones *et al.* reported that EBV had infected T lymphocytes and contributed to T-cell lymphoma in three patients.⁵⁾ Similarly, Kikuta *et al.* demonstrated EBV genome-positive T lymphocytes in a patient with chronic active EBV infection.¹⁵⁾

In the present study, we were able to demonstrate an additional example of T-cell lymphoproliferative disease observed in a patient with chronic active EBV infection. Our data indicate that EBV can infect T lymphocytes and might contribute to not only T-cell lymphoma but

also clonal T-cell lymphoproliferation. It seems noteworthy that all five reported cases had a phenotype of CD3⁺, CD4⁺ and HLA-DR⁺. This finding suggests that T-cell lymphoproliferative disease showing a phenotype of CD3⁺, CD4⁺ and HLA-DR⁺ might be related to EBV.

It is still unclear whether or not EBV induces the T-cell lymphoproliferative disease. To further elucidate the relationship between EBV infection and T-cell lymphoproliferative disease, more cases should be studied. It is likely that there might be a new subtype of T-cell

malignancies which results from EBV infection, just like African Burkitt's lymphoma, which is an etiologically and phenotypically specific subtype of B-cell malignancies.

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