Full Presence of Epstein-Barr Virus (EBV)-Encoded Latent Proteins in Tissues from a Patient with Severe Chronic Active EBV Infection Syndrome

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Epstein-Barr virus (EBV) is a causative agent for infectious mononucleosis and lymphoproliferative disorders (LPD) which occur in individuals with primary or acquired immunodeficiency, and is etiologically linked to the human malignancies such as EBV genome-positive Burkitt's lymphoma and nasopharyngeal carcinoma [1, 2]. Recent studies have disclosed that the EBV genome encodes at least 9 latent proteins and 2 RNAs. These are: EBV-determined nuclear antigens (EBNA)-1, -2, -3, -4, -5 and -6, latent membrane proteins (LMP)-1, -2A and -2B, and EBV-encoded RNA (EBER)-1 and -2. EBERs are expressed in all latently EBV-infected cells. Interestingly, expressions of latent proteins in tissues from the EBV-associated spectrum of diseases are different [1, 2]. Tissues from EBV genome-positive Burkitt's lymphoma generally express only EBNA-1, whereas LMPs and EBNA-1 are expressed in tissues from patients with nasopharyngeal carcinoma. In contrast, though heterogeneous EBV-latent gene expressions were recently reported in LPD with organ transplant recipients [3], tissues from LPD occurring in immunocompromised individuals usually express all EBV-encoded latent proteins. Additionally, EBV-specific cytotoxic T lymphocytes (CTL) are able to target these proteins except for EBNA-1 [4]. These observations suggest that different cellular expressions of EBV-related latent proteins and/or defective immunosurveillance may be highly associated with the development of a wide spectrum of diseases.

Severe chronic active EBV infection syndrome (SCAEBV) is an enigmatic but distinct syndrome, characterized by intermittent fever, lymphadenopathy, and hepatosplenomegaly [5]. Laboratory findings show a tendency to pancytopenia and polyclonal hypergammaglobulinemia. Extremely elevated antibody titers against EBV-replicating antigens such as viral capsid antigens and early antigens are commonly noted in this syndrome, and EBV genomes are consistently detected in each affected lesion. Furthermore, patients with SCAEBV often develop B cell- or T cell-originated LPD. Understanding the pathogenetic mechanism(s) for SCAEBV, especially regarding cellular immune functions, is thought to be important. However, these mechanisms remain unknown because the establishment of EBV genome-positive lymphoid cell lines (useful

for the analysis of EBV-specific CTL) is usually difficult owing to the observation that cytopathic effects occur in cultured lymphoid cells from these patients. Heterogeneous immunodeficiencies, including a lack of natural killer cell activity and/or EBV-specific CTL deficiencies, have been reported in this syndrome [5].

For these reasons, evaluation of the expression levels of EBV-related latent proteins in tissue is considered to be one of the keys for understanding the development of this disease. We have investigated the expression of these EBV-related latent proteins in different tissues from a patient with SCAEBV.

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The patient, a 24-year-old male, was described precisely elsewhere [6]. Briefly, he suffered from intermittent fever, supraclavicular, bilateral axillary and inguinal lymphadenopathy and hepatosplenomegaly from the age of 23 years. He had increased serum levels of IgM, IgG and IgA, and pancytopenia during the observation period. The resected spleen showed oligoclonal rearrangement of the heavy-chain J-region fragment of the immunoglobulin gene, and contained EBV genome. Pathological findings showed marked cordal lymphocytosis and plasmacytosis. Lymph node specimens showed slight paracortical lymphoid depletion and a few scattered granulomata. There was no evidence of malignant lymphoma in tissues examined. He finally developed massive lower gastrointestinal bleeding and expired. Attempts to establish EBV-positive lymphoid cell lines from each affected lesion failed. Therefore, EBV-specific CTL activities were not evaluated.

Each tissue from the resected spleen and a paranesenteric lymph node from autopsy samples of the patient, was admixed with 0.065 M Tris-HCl (pH 6.8), 5% beta-mercaptoethanol, 3% SDS, 10% glycerol and 0.01 % bromophenol blue, and was electrophoresed and transferred onto a nitrocellulose filter paper as described elsewhere [7]. The nitrocellulose filter paper was stained with polyvalent human serum containing high IgG antibody titers against each EBNA (except for EBNA-5) in blocking buffer, and incubated with alkaline phosphatase-conjugated goat antihuman IgG. LMP-1 was detected using the S12 monoclonal antibody (kindly provided by Dr. D. Thorley-Lawson, Boston, Mass., USA), and alkaline phosphatase-conjugated goat antimouse IgG used as a second antibody.

As shown in table 1, each tissue sample, that was positive for EBV DNA, was evaluated for the presence of EBV-related latent proteins. EBNA-1, -2, -3, -4 and -6 were positive in the spleen and paranesenteric lymph node by immunoblotting procedures. LMP-1 was also positive for each tissue using monoclonal antibodies. These data indicate that fresh tissue samples from a patient with SCAEBV express all of the EBV-related latent proteins.

Table 1. Presence of EBV-encoded latent proteins in tissues from a patient with SCAEBV

Latent protein

Spleen

Paranesenteric lymph node

EBNA-1

+

+

EBNA-2

+

+

EBNA-3

+

+

EBNA-4

+

+

EBNA-5

unknown³

unknown

EBNA-6

+

+

LMP-1

+

+

^a Presence of EBNA-5

was not evaluated because the serum used in this study did not contain sufficient anti-EBNA-5 antibodies.

Recently, Japanese investigators have reported that they established a spontaneous lymphoblastoid cell line from a patient with SCAEBV, and EBV-related latent proteins for EBV-specific CTL were demonstrated to this cell line [8]. However, some Burkitt's lymphoma cells expressing only EBNA-1 in tissue were reported to express all EBNAs and LMPs during the course of cultivation [9]. Therefore, these data suggest that it is important to assess the level of EBV-related latent protein expression in tissue.

These observations suggest that the pathogenetic mechanism(s) for SCAEBV are mainly related to defective or imbalanced cellular immunosurveillance. Therefore immunomodulation may be beneficial in treating this enigmatic syndrome.

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