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Case of Leukemic Changes in Hepatosplenic Diffuse Large B-Cell Lymphoma (DLBCL) in a Human T Lymphotropic Virus Type 1 (HTLV-1) Antibody-Positive, HCV-Negative Patient

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Abstract A 78-year-old female developed lymphocytosis and splenomegaly. Antibodies to human T-lymphotropic virus type 1 (HTLV-1) were positive; however, incorporation of HTLV-1 proviral DNA was negative. Hepatitis B virus surface antigen (HBsAg) and hepatitis C virus (HCV) antibody were negative. Flow cytometry of the lymphocytes revealed the B-cell phenotype, and rearrangement of the immunoglobulin heavy chain gene was positive. She was treated with cladribine administration and splenic radiation, which were ineffective and she died a month later. Autopsy liver findings revealed infiltration of atypical lymphocytes, which were immunohistochemically positive for CD20, while the spleen revealed diffuse infiltration of atypical lymphocytes which were positive for CD20, Bcl-2 and cytoplasmic (C) Igκ. Some of them were positive for MIB-1 and MUM1. These results indicated leukemic changes in a hepatosplenic diffuse large B-cell lymphoma (DLBCL) with plasma cell differentiation. Lymphoma cells were negative for EBV-encoded latent membrane protein 1 (LMP-1) and EB nuclear antigen-2 (EBNA2) by immunohistochemistry and for EBV-encoded mRNA 1 (EBER-1) by in situ hybridization. The cause that may have contributed to the pathogenesis of hepatosplenic DLBCL is unknown.

Key words: human T-lymphotropic virus type 1, carrier, hepatosplenic diffuse large B-cell lymphoma, Epstein-Barr virus, hepatitis virus

Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) infection is directly linked to the pathogenesis of adult T-cell leukemia/lymphoma (ATL/L) via the incorporation of HTLV-1 into T-lymphocytes and their subsequent transformation. There are several subdivisions of the disease; acute, chronic, smouldering, lymphoma (ATL/L), and antibody-positive carrier without disease onset. While the HTLV-1 seroprevalence rate in patients with other malignancies besides ATL/L is reported to be higher than in control individuals, HTLV-1 infection increases the risk of other hematological and non-hematological malignancies.[1-4] Infection by HTLV-1 or by infection with other pathogens may contribute to the pathogenesis of malignancies other than ATL/L during disturbed immunosurveillance.[1-4] We report a case of hepatosplenic diffuse large B-cell lymphoma (DLBCL) in a patient positive for the HTLV-1 antibody, that is a carrier. No relationship between Epstein-Barr virus (EBV) infection and the hepatitis virus for the pathogenesis

of malignant lymphoma in the present patient was found despite analysis.

Case report

A 78-year-old female complained of general dullness and fever from middle of June, 2005. CT demonstrated huge splenomegaly. Laboratory data are presented in Table 1. CBC revealed anemia and thrombocytopenia. Atypical cells in the peripheral blood were mainly large immature lymphocytes which were negative for peroxidase, esterase and PAS stainings, but a small % of lymphocytes with lobulated nuclei were also observed (Fig. 1). She was referred to our hospital in the middle of August. Physical examination on admission revealed anemia and splenomegaly, and the superficial lymph nodes were not palpable. A liver function test showed slightly elevated levels of total bilirubin, AST, ALT and LDH. The hepatitis B virus surface antigen (HBsAg) and hepatitis C virus (HCV) antibody were negative.

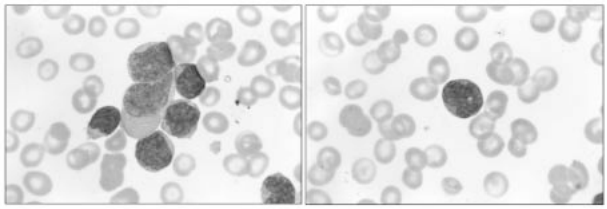

Fig. 1 A peripheral blood smear shows large immature lymphocytes (left) and lymphocytes with multilobulated nuclei (right).

Flow cytometric analysis of atypical lymphocytes in the peripheral blood were positive for CD5, CD19, CD20 and surface immunoglobulin (Ig) κ-chain, and negative for CD11c and CD23. Atypical lymphocytes were negative for tartrate resistant acid phosphatase staining.

The level of soluble IL-2 receptor was markedly elevated. A bone marrow aspiration smear revealed 11.6% atypical lymphocytes, and results from the flow cytometric analysis of bone marrow cells were similar to those of peripheral blood, while chromosomal analysis of bone marrow cells indicated 46, XX. No hemophagocytic histiocytes were observed in the bone marrow smear. The levels of serum immunoglobulin (Ig) were normal. The HTLV-1 virus antibody was positive. Incorporation of HTLV-1 proviral DNA in the lymphocytes was negative, and rearrangement of the Ig heavy chain (IgH) gene was positive (Fig. 2) by Southern blot hybridization. Epstein-Barr virus (EBV) genomic DNA was positive by polymerase chain reaction (PCR) in the peripheral blood cells.

Table 1 Laboratory data on admission

CBC	
RBC (10^4/μl)	289
Ht (%)	25.7
Hb (g/dl)	8.5
Plt (10^4/μl)	1.8
WBC (/μl)	6600
Neutro (%)	37
Lymph (%)	25
Mo (%)	16
Atyp Cell (%)	18
Erythroblast (%)	6
Flow Cytometry	
CD5 (%)	63
CD19 (%)	96
CD20 (%)	92
κ-chain(%)	62
Bone Marrow	
Atyp Cell (%)	12
Chromosome	46,XX
Liver Function	
Total Bil (mg/dl)	1.2
AST (IU/l)	152
ALT (IU/l)	34
LDH (IU/l)	1029
Immunoglobulin	
IgG (mg/dl)	1480
IgA (mg/dl)	272
IgM (mg/dl)	51
Serology	
HBs Ag	negative
HCV Ab	negative
HTLV1-1 Ab (x)	256
S-IL2-R (U/ml)	20500

Clinical course (Fig. 3)

The patient was administered cladribine; however, it was stopped since the ATL was considered substantial. Meanwhile, leukocytes and atypical lymphocytes increased to 34,000/μl, and the patient complained of abdominal pain due to splenomegaly. Restarting cladribine was considered, but the patient and family refused, so administration of prednisolone and radiation to the spleen were performed. The leukocyte count dropped to 10,100/μl, but her general condition deteriorated, and radiation was stopped (total irradiation dose, 12Gy). The leukocyte count increased again after ceasing radiation, reaching 30,000/μl. The patient died in the middle of September, and autopsy was performed. The liver showed patchy infiltration of large atypical lymphocytes in the dilated sinuses and many large atypical lymphocytes in the portal areas, which were immunohistochemically positive for CD20. These results indicated invasion by malignant lymphoma of a large diffuse cell type. The spleen, weighting 800g, revealed massive coagulative necrosis and diffuse infiltration of large atypical lymphocytes with round nuclei which were positive for CD20, CIgκ, Bcl-2, and 30% of them were positive for MIB-1 and MUM1, and negative for CD3, CD5, CD10, Bcl-1, Bcl-6, CD23, CD25, CD30, and cyclin D1. These results indicated invasion by a diffuse large B-cell lymphoma. Based on these reults, the diagnosis of leukemic changes in hepatosplenic diffuse large B-cell lymphoma (DLBCL)

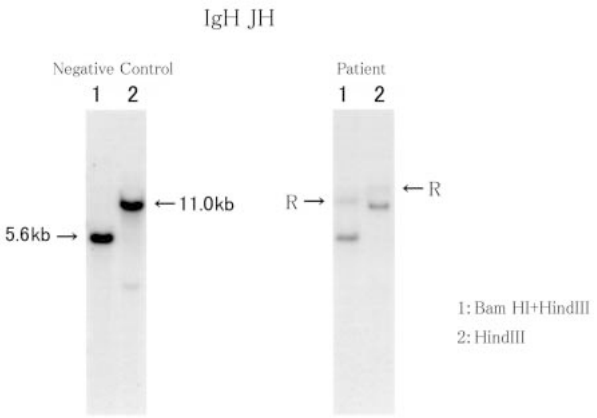

Fig. 2 Southern blot analysis of the blood showing rearrangement (R) of the immunoglobulin heavy chain (IgH) gene.

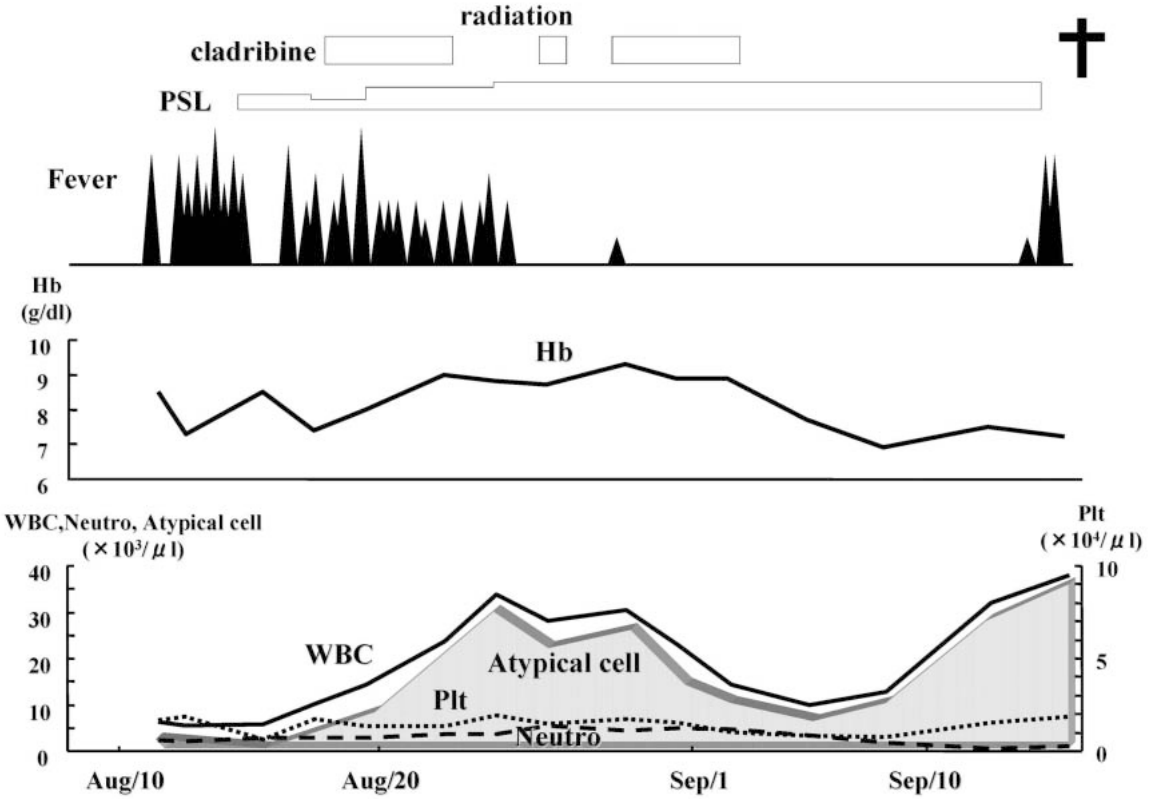

Fig. 3 The clinical course. PSL:prednisolone

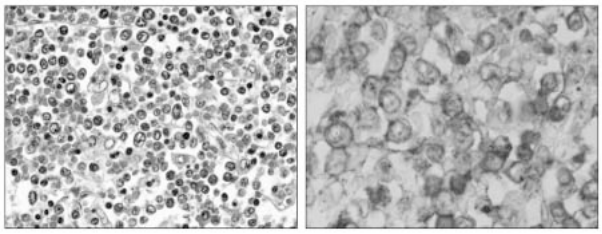

Fig. 4 Microscopic findings of the spleen autopsy specimen showing diffuse infiltration of large immature lymphoblasts (left) which were positive for CD20 (right) staining diagnosed as diffuse large B-cell lymphoma (DLBCL).

with plasma cell differentiation was made (Fig. 4).

These cells were negative for EBV-encoded latent membrane protein 1 (LMP-1) and EBNA2 by immunohistochemistry, and for EBV-encoded mRNA 1 (EBER-1) by in situ hybridization. They were negative for herpesvirus 8 (HHV8), as confirmed by immunohistology for HHV8-encoded protein latent nuclear antigen (LNA).

Discussion

Asou et al. found a high frequency of malignancy was observed in patients with smouldering ATL patients (five of 18 cases), and subsequently they investigated the relationship between HTLV-1 infection and other malignancies, reporting that 61 of 394 patients (15%) with malignancies were positive for the HTLV-1 antibody.[1] Ono et al. reported a significantly higher superimposed rate of cancer in ATL patients; five of 43 patients (11.6%), in contrast to the cancer prevalence rate of three of 155 subjects (1.9%) with HTLV-1 seronegative hematological malignancies.[2] Imamura et al. reported that the incidence of multiple primary neoplasms in ATL patients was higher, at 33%, compared with patients with other hematologic malignancies at 4%.[3] Kozuru et al. reported that the incidence of primary malignant neoplasms was higher in patients with ATL/L than the occurrence in HTLV-1 seronegative non-Hodgkin's lymphoma (NHL) patients, and the incidences were also higher in the siblings and mothers than other family members.[4]

Various kinds of neoplasms in various states of HTLV-1 infection have been reported, including non-hematological neoplasms, and cancers of various organs, in HTLV-1 carriers and ATL patients,[1-4] and hematological neoplasms, multiple myeloma,[5] and acute myeloblastic leukemia[6)9] in ATL patients, B-cell lymphoma and early gastric cancer in a smouldering ATL patients,[7] B-cell lymphoma of a localized stage of the neck and head in HTLV-1 carriers,[8] and Burkitt's lymphoma, in HTLV-1 carriers.[9] Thus, the majority of lymphocytic neoplasms were of B-cell origin, except for ATL/L cases. In the present patient, leukemic changes in hepatosplenic B cell lymphoma with plasma cell differentiation was diagnosed.

Lymphocyte transforming viruses such as EBV, HHV8, and HTLV-1 directly infect a subset of lymphoid cells in which they express viral oncogenes.[10] Infection with EBV has been implicated in the development of a variety of lymphoproliferative malignancies[11] and B-cell lymphoma,[12] as proved by Southern blot analysis, polymerase chain reaction (PCR), or EBV-encoded mRNA 1 (EBER1) in situ hybridization. However, the incidence of positive EBV expression was unexpectedly low, (12-24%), depending on the types of examination, in B-cell lymphoma unless patients were immunologically impaired, such as those post-organ transplantation or with autoimmune diseases.[12]

There are several reports indicating the role of EBV infection in the pathogenesis of B-cell lymphoma in ATL/L patients and HTLV-1 carriers and EBV genome was found to be positive in B-cell lymphoma cells in a patient with ATL/L,[13] EBV-associated Hodgkin's disease was reported in two HTLV-1 seropositive patients[14] and a high rate of detection of EBV and HTLV-1 in malignant lymphoma was observed in Okinawa, which is an area endemic for ATL.[15] In the present patient, the direct role of EBV infection in the pathogenesis of B-cell lymphoma was ruled out. Genomic DNA was detectd by PCR in the peripheral blood, but the significance of this result remains unclear.

There are several reports of B-cell lymphoma in HCV- positive patients; 1) non-

Hodgkin's lymphoma (NHL) frequently involving extranodal sites such as in the liver, salivary glands and spleen,[16] 2) primary splenic lymphoma[17)18)] and 3) primary hepatosplenic lymphoma.[19] Fifty two percent of splenic DLBCL cases were positive for HCV antibodies, and the prevalence was significantly higher than the 9.3 percent of nodal DLBCL patients.[18] Twelve of 54 B-cell non-Hodgkin's lymphoma (NHL) cases were positive for HCV antibodies, which was higher than in non-B-cell NHL, and of these four were diagnosed as primary hepatosplenic DLBCL, which is rare in NHL since most cases are of T-cell origin.[19] Splenic lymphoma usually has a lobulated tumor mass.[17)18)] The present case is rare in that hepatosplenic DLBCL was not associated with hepatitis virus infection, and no tumor mass was observed in the spleen. Hemophagocytic syndrome (HPS) in patients with EBV-negative B-cell lymphoma has been reported.[20)21)] The majority of lymphoma-associated HPS has been observed in the T-cell/natural killer cell (T/NK-cell) phenotype.[22] These T/NK-cell lymphomas with HPS were shown to be infected with clonal EBV, as well as reactive T-cells, in virus-associated hemophagocytic syndrome (VAHS).[22] In the present patient, HPS, as well as EBV infection, were not observed.

HTLV-1 infected helper and cytotoxic T cells have altered functions in vitro.[1)3)5)8)] The altered immunosurveillance had been considered that may contribute to the oncogenesis of other neoplasms in ATL/L patients and HTLV-1 carriers.[1)3)5)8)] However, the cause that complicated B-cell hepatosplenic lymphoma in the present patient is unknown, since the levels of serum immunoglobulins were normal and cellular immunity was not studied in the present patient.

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