

Epstein-Barr Virus-Associated Peripheral T-Cell Lymphoma involving Spleen in a Renal Transplant Patient

The incidence of posttransplantation lymphoproliferative disorders (PTLDs) has increased in recent years. Although rare, various types of T-cell lymphoma have been reported and their association with Epstein-Barr virus (EBV) has been compared with B-cell PTLDs. We report a case of splenic peripheral T-cell lymphoma occurring in a 47-yr-old male patient 7 yr after renal allograft transplantation. The spleen showed sinusoidal proliferation of focal CD30 positive, large, atypical lymphoid cells. Positivity for CD3 and cytolitic granule-associated proteins was also demonstrated in the tumor cells, while anaplastic large cell lymphoma kinase (ALK) and CD8 were not expressed. Strong nuclear signals for EBV mRNA were noted by EBER1 in situ hybridization. A molecular genetic study demonstrated a rearrangement of the gamma T-cell receptor gene. To our knowledge, this case is unique in terms of a posttransplant T-cell lymphoma that shows focal CD30, cytolitic granule-associated proteins, and EBV positivity.

Key Words : Lymphoproliferative Disorders; Lymphoma, T-Cell; Spleen; Herpesvirus 4, Human; Antigens, CD30; Antigens, CD3

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INTRODUCTION

Both the newly available powerful immunosuppressive agents and the steady increase of the number of organ transplantation have increased the incidence of posttransplantation lymphoproliferative disorders (PTLDs) in recent years. Most PTLDs are B-cell derived and are closely linked to Epstein-Barr virus (EBV). It is estimated that 14% of PTLDs are of T-cell origin and over 60 cases of posttransplant T-cell malignancies have been reported so far (1-3). The most common type of PTLDs of T-cell origin is peripheral T-cell lymphoma, and in contrast to the B-cell PTLDs, the association with EBV is less constant in posttransplant T-cell lymphomas. We herein present the clinical, histological, immunophenotypic, and molecular features of a focal Ki-1-positive, anaplastic large cell lymphoma kinase (ALK)-negative T-cell lymphoma that occurred in a patient receiving long-term immunosuppressive therapy after renal transplantation.

CASE REPORT

A 47-yr-old male patient received a cadaveric renal transplant in July 1991 at the age of 40 yr for chronic renal failure of 10 yr. His immunosuppression regimen consisted of azathioprine, prednisolone, and oral cyclosporine. In May 1997, splenomegaly was noted on ultrasonography. In January 1998, he presented with a general ache, mild fever, recurrent paranasal sinusitis, and enlargement of multiple right cervical lymph nodes which measured up to 1.5 cm in diameter. Physical examination revealed enlarged tonsils with a whitish plaque. A computerized tomography scan revealed numerous enlarged bilateral cervical lymph nodes and variable-sized multiple masses in the spleen without definite abnormality in the liver. A cervical node biopsy was performed and the diagnosis of a malignant T-cell lymphoma was made. The dosage of cyclosporine was adjusted by monitoring the whole blood cyclosporine level and was in the range of 125 ng/mL. He received whole neck radiation therapy with 4.14 Gy from January 1998 to February 1998. Laboratory studies

showed the followings; hemoglobin, 9.0 g/dL; hematocrit, 25%; WBC count, $1.2 \times 10^9/L$; and platelet count, $6 \times 10^9/L$. Examination of peripheral blood smear showed microangiopathic hemolytic anemia; burr cells, helmet cells, along with immature granulocyte precursors and toxic granules of mature granulocytes. Bone marrow biopsy showed occasional hemophagocytic histiocytes without atypical lymphocytes. Results of the serologic study for virus were as follows: Epstein-Barr virus viral capsid antigen (VCA) IgG: >1:160, VCA IgM: negative, EBNA: positive, EBV DNA PCR: positive, EBV early antigen (EA)-DR IgG: positive, EA-DR IgA: negative, and EA-DR IgM: positive. There were a diffuse pattern of EA-D both in the nucleus and cytoplasm and a restricted pattern of EA-R in the cytoplasm. Other viral test were cytomegalovirus IgG/IgM (+/-), herpes virus IgM: negative, hepatitis C virus antibody: negative, human immunodeficiency virus antibody: negative, and anti-platelet antibody: negative. In May 1998, a diagnostic and therapeutic splenectomy was performed because of a diagnostic impression of thrombotic thrombocytopenic purpura. After splenectomy, he suffered a postoperative intracerebral hemorrhage and died. Autopsy was not performed.

Methods of Laboratory Investigations

Routine morphologic studies were done on 4- μ m tissue sections fixed in formalin, and stained with hematoxylin and eosin. Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections of the spleen using a labeled streptavidin-biotin method (Universal LSAB kit, DAKO, Carpinteria, CA, U.S.A.). All antibodies were obtained from commercial sources. The primary antibodies were CD3, CD20, CD30, CD43, CD45RO, CD68, ALK (Dako, Carpinteria, CA, U.S.A.), CD4, CD5, CD8, CD56, granzyme B (Novocastra, Newcastle-upon-Tyne, U.K.), TIA-1 (Immunotech, Miami, FL, U.S.A.), and TCR α/β (T cell Diagnostics, Cambridge, MA, U.S.A.). We performed the in situ hybridization to detect and localize EBV genomes using a biotinylated 30-base oligonucleotide probe complementary to a portion of the EBV EBV1 gene, which is a gene of the EBV genome that is actively transcribed (up to 10^7 copies/cell) in latently infected cells.

Serologic tests for IgG and IgM antibodies to EBV-VCA (BION, Illinois, U.S.A.), EBNA (BION, Illinois, U.S.A.) and IgG, IgA, and IgM antibodies to EBV EADR (Granbio, California, U.S.A.) were performed by immunofluorescent antibody assay. The patient's blood was screened for EBV DNA using the Bam W primers and 35 cycles of PCR amplification. The sequences of the primers and probe used were as follows:

TC60 primer: CCAGAGGTAAGTGGACTT

TC61 primer: GACCGGTGCCTTCTTAGG

TC62 probe: TTCTGCTAAGCCCAAC

For genotyping, DNA was extracted from the paraffin-

embedded spleen. The V and J regions of the TCR- γ chain primer were used for TCR- γ gene rearrangement amplification using GeneAmp 9600 (4). Nested PCR was first performed with V_A and J_A primers and the second round was performed with V γ and J γ primers. The PCR products were denatured and then renatured on ice for 20 min. The products were electrophoresed on a 15% polyacrylamide gel at 12°C and developed with silver staining. The sequences of primers for TCR γ (5'→3') were as follows:

V_A: 5'-GAAGCTTCTAGCTTTCCTGTCTC-3'

J_A: 5'-CGTCGACAACAAGTGTGTTCCAC-3'

V γ : 5'-AGGGTTGTGTTGGAATCAGG-3'

J γ : 5'-CGTCGACAACAAGTGTGTTCCAC-3'

Pathologic findings

The cervical lymph node showed diffuse effacement of the structure by medium-sized lymphocytes having irregular nuclear outlines as well as immunoblasts (Fig. 1). The atypical lymphoid cells demonstrated positivity for CD3 and TIA-1, focal positivity for CD30, and negative reaction to CD20 and CD79a on the immunohistochemical study. The spleen measured 20 × 10 × 5 cm and weighed 550 g. Cut sections revealed multifocal, poorly defined pinkish-brown nodular masses measuring up to 7 cm in diameter (Fig. 2). Histologic sections of the spleen revealed a diffuse red pulp infiltrate of large lymphoid cells with a near-total effacement of the white pulp structure. The tumor tissue was diffusely infiltrated by large-sized lymphoid cells with irregular, often multilobated vesicular nuclei with one or more prominent nucleoli and extensive pale to basophilic cytoplasm (Fig. 3A). Mitotic figures were frequently encountered. In the congested red pulp, there were also numerous banal-looking histiocytes showing hemophagocytosis (Fig. 3B). The large atypical lymphoid cells demonstrated an aberrant T-cell immunophenotype (CD3+, CD4-, CD5-, CD8-, CD43+, and CD45RO+) in the immunohistochemical studies. The tumor cells demonstrated cytoplasmic positivity for TIA-1, CD30, and granzyme B (Fig. 4). Neither ALK nor TCR α/β expression was observed. The hybridization signal for the EBV EBV1 RNA probe was detected in more than 95% of the large atypical neoplastic cells in both cervical lymph node and the spleen (Fig. 5). PCR DNA analysis using the splenic tissue showed a clonal rearrangement of the TCR- γ chain gene (Fig. 6). DNA study and immunostain of the lymph nodes were not done.

DISCUSSION

PTLDs are a heterogeneous group of lymphoid proliferative disorders that are distinguished by specific histological, phenotypic, and genotypic features. Non-Hodgkin's lymphoma is the most common lymphoma that occurs in transplant recipients (TRs) accounting for 21% of all malignant neo-

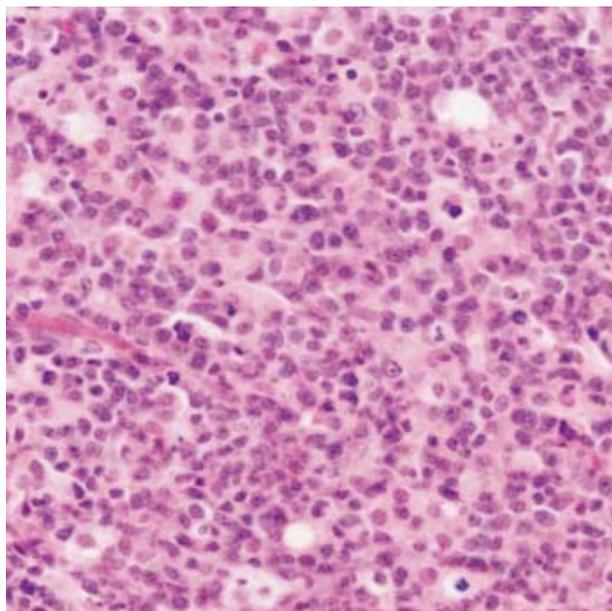


Fig. 1. Lymphoma cells are intermediate to large in size and have oval-shaped nuclei with irregular contour, coarse chromatin, and moderate amount of pale cytoplasm (H&E, $\times 200$).

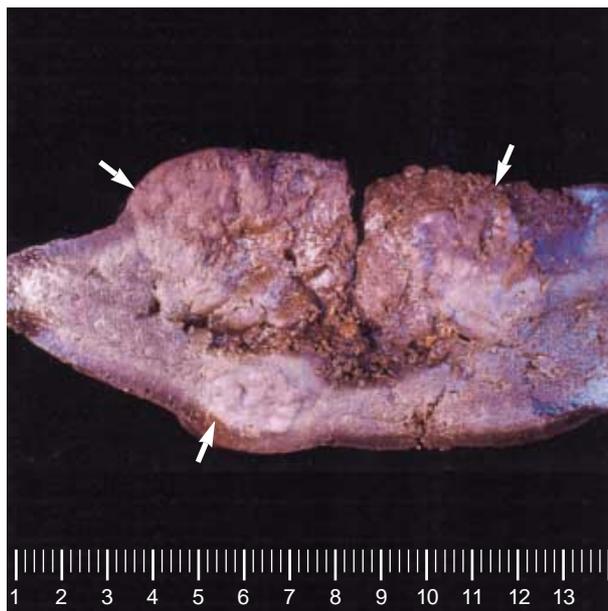


Fig. 2. Cut section of the spleen reveals multifocal ill-defined red-brown nodular masses (arrows).

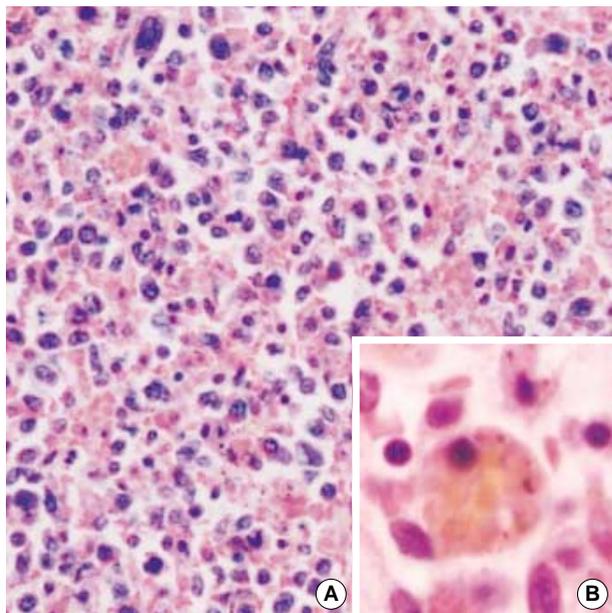


Fig. 3. The splenic red pulp is diffusely infiltrated with an abnormal population of lymphoid cells showing irregular large nuclei (A) (H&E, $\times 200$). Some histiocytes showed erythrophagocytosis (B) (H&E, $\times 400$).

plasias. Most of these lymphomas are classified as large-cell lymphomas, the great majority of which are of the B-cell type. However, non-B-cell lymphomas also occur in TRs, with 14% being of T-cell origin (1-3). Like T-cell lymphomas that occur in immunocompetent patients, the majority are

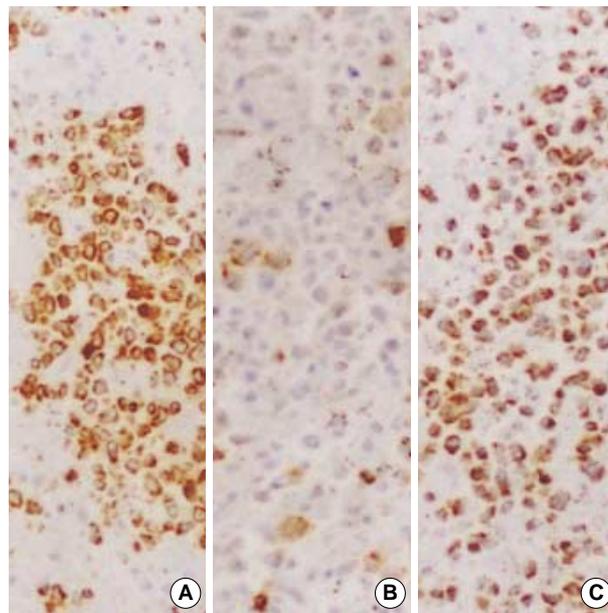


Fig. 4. Immunohistochemical demonstration of TIA-1 (A), CD30 (B), and granzyme B (C) in the tumor tissue (PAP, $\times 100$).

peripheral T-cell lymphomas, not otherwise specified. These T-cell lineage lymphomas, like most peripheral T-cell lymphomas (PTCL), commonly have either a T-helper (CD4+) or a T-cytotoxic (CD8+) phenotype and express the α/β T-cell receptor heterodimer. CD8 is more commonly expressed by α/β T cells, but CD8 can also be expressed by some γ/δ T-cell lymphomas. Interestingly, our patient showed a γ chain

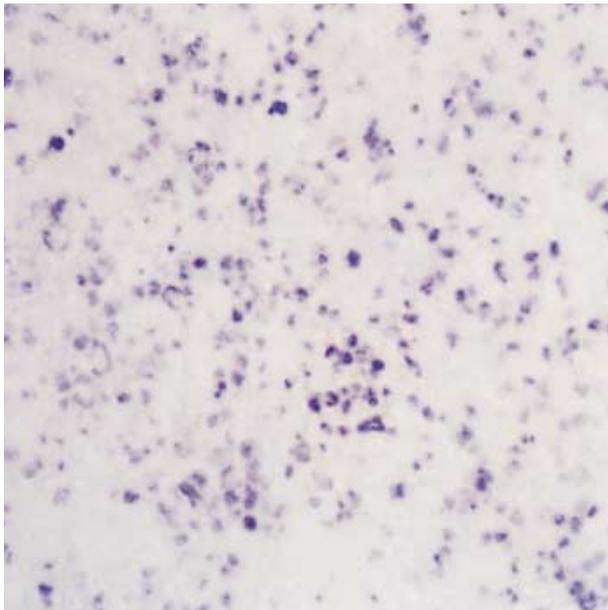


Fig. 5. Diffuse positivity for EBV on in situ hybridization using the EBV EBER1 RNA probe ($\times 100$).

TCR gene rearrangement with an expression of TIA-1 and granzyme B, indicating activated cytotoxic T-cells phenotype without definite immunoreactivity of CD8. Recently, several studies have shown that the expression of these cytotoxic proteins in PTCL tumors is associated with an extranodal site, a T gamma delta-cell phenotype, CD30 expression, and anaplastic feature (5). Rothenberg et al. (6) mentioned an immune reaction of cytotoxic $\gamma\delta$ T lymphocytes in an EBV-induced PTLD. The cytotoxic T cells predominantly expressed $\gamma\delta$ TCR rather than $\alpha\beta$ TCR and mediated non-major histocompatibility-restricted cytotoxicity against EBV-infected cells. Under these circumstances, although we can not apply this suggestion to our case definitely, we think that the cytolytic granule-associated proteins may suggest cytotoxic T lymphocyte differentiation in spite of the negative immunoreactivity of CD8. Also, EBV is known to induce the CD30 expression in EBV-transformed cell lines; therefore EBV-associated posttransplant lymphomas may prove to be CD30+.

As differential diagnosis, we considered the possibility of anaplastic large cell lymphoma (ALCL) and $\gamma\delta$ hepatosplenic T-cell lymphoma. Ki-1-positive ALCL is a subtype of PTCLs showing CD30 immunoreactivity and only a few cases of Ki-1-positive B-cell lymphoma have been reported as PTLD. However, with the focal CD30 expression, it might be improper to diagnose the present patient as ALCL without showing ALK positivity. We also considered the possibility of $\gamma\delta$ hepatosplenic T-cell lymphoma. The findings of the γ chain TCR gene rearrangement, the expression of TIA-1, and the negative reactions for both CD4 and CD8 have been described but a positive expression of CD30 and granzyme

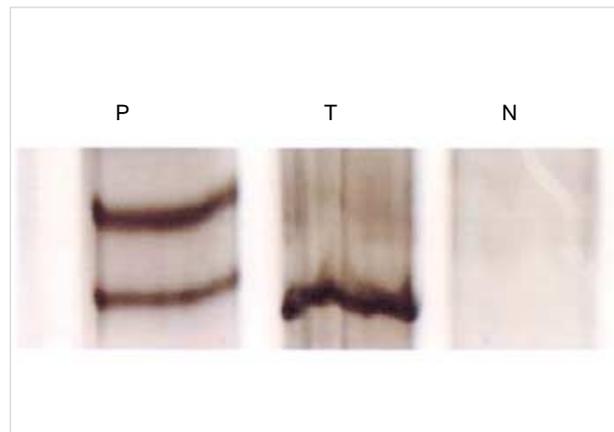


Fig. 6. Polymerase chain reaction (PCR) analysis for T-cell receptor V-gamma-J-gamma reveal a clonal V-gamma-J-gamma rearrangement (lane T). Lane N is the negative control and lane P is the positive control.

B are not features of $\gamma\delta$ hepatosplenic T-cell lymphoma.

Lymphoproliferative disorders occurring in association with immunosuppression are unique. Of concern is the role of EBV in the pathogenesis of these EBV-associated PTLDs. Compared with B-cell PTLDs, T-cell PTLDs show a looser association with EBV and more often monoclonality. EBV infection was believed to be limited to B lymphocytes, follicular cells of lymph nodes and tonsils, and epithelial cells of the pharynx and cervix. B lymphocytes are known to be infected via the C3d receptor (CD21), which is expressed constantly in benign and malignant B cells. However, recent reports have shown that CD21 and EBV may be present in some malignant T-cells. Medeiros et al. (7) suggested that EBV may be involved in the transformation of low grade T cells proliferation to high grade lesions, and Huh et al. (8) described the high incidence of EBV in peripheral T cell lymphomas in Koreans. However, the exact mechanism of how the EBV get into the T-cells is still unclear. Because most B-cell PTLDs are primary EBV infections, a lack of previous EBV infection is a risk factor of B-cell PTLDs. Conversely, chronic EBV infection was assumed to be the cause of EBV-associated T-cell lymphoma in the previous reports. All 10 cases of EBV-associated T-cell lymphomas reported by Su et al. (9) had evidence of previous infection, and none of them had an elevated level of IgM class anti-VCA. In our patient, IgM and IgG EA-DR were positive. This suggests reactivation of a past EBV infection. We could not find a report of T-cell PTLD with IgM positivity in the literature.

Clinically, at the time of diagnosis, most of the T-cell PTLD cases had fully developed T-cell malignant neoplasms. The long-term survival of patients with T-cell PTLD is generally poor, as was shown in the present case and in others. Most of the patients died within 1 yr from the diagnosis. In view of high mortality rate of this lymphoma, early diagnosis is critical to the patients' outcome.

To our knowledge, this is the first case report of a post-transplant T-cell lymphoma involving spleen, which is associated with EBV positivity, gamma TCR gene rearrangement, cytolytic granule-associated proteins, and CD30 positivity.

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