Journal of Korean Medical Science
Vol. 9, No. 2, 162-168 April 1994

Epstein-Barr Virus Associated Posttransplant Malignant Lymphoma in Renal Allograft Recipients

Yeong Jin Choi, M.D., Chang Suk Kang, M.D.,
Wan Shik Shin, M.D.,* Mi Kyoung Jee, M.D., Byoung Kee Kim, M.D.,
Sun Moo Kim, M.D., Sang In Shim, M.D.

Department of Clinical Pathology
and Internal Medicine, Catholic University Medical College, Seoul, Korea*

We report two cases of posttransplant malignant lymphoma(PTML) of B cell origin associated with Epstein-Barr virus(EBV) infection. They were a 52-year-old male and a 37 year-old-female, in whom intermediate-grade diffuse malignant lymphomas of large cell type developed in the submandibular area and jejunum, respectively. DNA and RNA in situ hybridization revealed the presence of EBV-specific DNA and RNA sequences in the tumor cells.

Key Words : *Posttransplant malignant lymphoma, Epstein-Barr virus, In situ hybridization.*

INTRODUCTION

Posttransplant malignant lymphoma(PTML) is a well-known complication in renal allograft recipients. It is almost always of B cell origin and is frequently associated with Epstein-Barr virus (EBV) infection in immunosuppressed patients(Hanto et al., 1982 ; Nalesnik et al., 1988 ; Starzl et al., 1984), which induces uncontrolled proliferation and mutation of B-lymphocytes resulting in malignant transformation. The time interval between transplantation and tumor detection varies from 0.7 months to 3 years, with a mean of about 5 months in cyclosporin-treated patients(Nalesnik et al., 1988). PTML may be single or multiple and often arises in the central nervous system, the gut, heart, skin and bone other than lymph nodes in contrast to non-Hodgkin's lymphoma. In this report, two cases of EBV-associated malignant lymphoma arising after transplantation with living-related donors are described.

Address for correspondence : *Yeong Jin Choi, M.D., Department of Clinical Pathology, Catholic University Medical College, Kangnam St. Mary's Hospital, 505 Banpo-Dong, Seocho-Ku, Seoul 135-040, Korea. Tel : (02) 590-1603*

CASE REPORT

Case 1

A 52-year-old male patient was admitted for a palpable mass in the left submandibular area for 2 months. He received a living-related renal allograft transplant from his son in March 1992. Immediately after kidney transplantation(KT), acute rejection developed and OKT3 monoclonal antibody was used for 5 days. Then renal function recovered and was maintained with cyclosporine-A and steroids. Five months after KT, a rapidly growing palpable mass was noted in the left submandibular area. Computed tomography of the neck revealed a large hyperdense mass, 6X6X5cm, in the left submandibular area (Fig. 1). In serologic tests for EBV infection, EBV anti-viral capsid antigen(VCA) IgG was 1 : 320, and EBV anti-early antigen(EA)- DR was less than 1 : 10 (Table 1). Antibodies for human immunodeficiency virus(HIV-1), cytomegalovirus and hepatitis B virus were all negative. A percutaneous needle biopsy of the mass was performed.

Case 2

A 37-year-old female patient received a living-

Table 1. Serologic markers

	Case 1	Case 2
EBV VCA IgG	1 : 320	1 : 80
EBV VCA IgM	ND	<1 : 10
EBV EA-DR IgG	<1 : 10	<1 : 10
EBV EBNA	ND	1 : 10
Anti-HIV	—	ND
Anti-CMV	—	ND

ND : not done

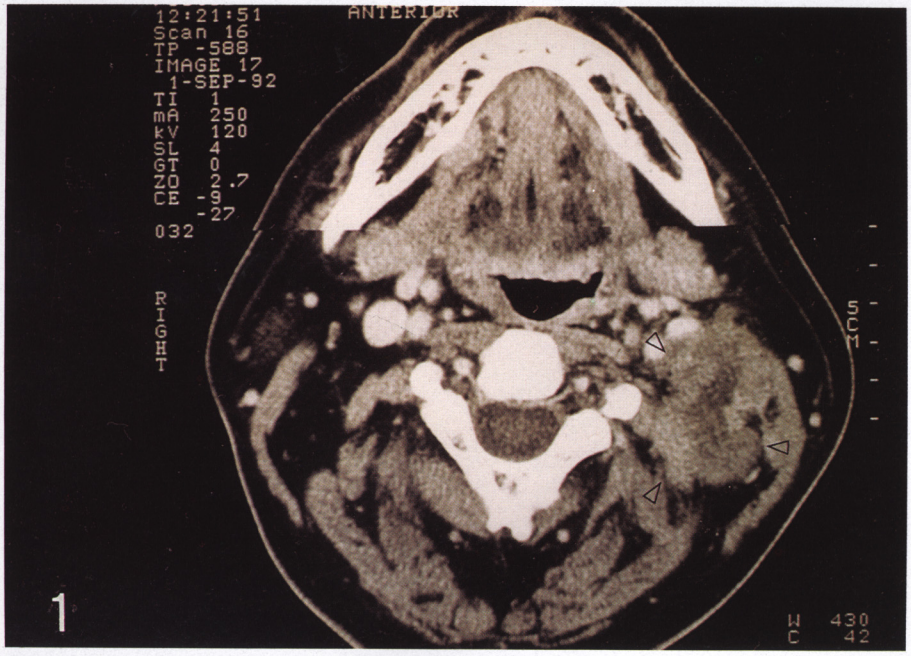

Fig. 1. Computed tomography of the neck reveals a large mass(arrowhead),6×6×5 cm, with central hypodense areas in the left submandibular area.

related renal allograft transplant from a relative. She had a history of renal tuberculosis and had received a right renal nephrectomy 20 years ago. During the follow-up period, she had been well without complication, except for a high level of cyclosporine A in the whole blood more than 1000 ng/ml. Five months after KT, acute abdominal pain developed and she was admitted to our hospital. On admission, she looked very ill and there was marked tenderness in the lower abdomen on palpation.

Serologic examination for EBV infection revealed anti-EA-DR IgG less than 1 : 10, anti-VCA IgG 1 : 80, anti-VCA IgM less than 1 : 10, and anti-EBV nuclear antigen 1 : 10(Table 1). Computed tomography of the abdomen revealed a large submucosal mass density in the jejunum. An exploratory laparotomy and segmental jejunal resection were done.

METHODS

The specimen was fixed in 10% buffered formalin, embedded in paraffin and stained with hematoxylin-eosin and periodic acid schiff. Immunostaining was performed on paraffin-embedded tis-

sues using avidin-biotin peroxidase technique. For in situ hybridization, procedures previously described by Lezzoni et al.(1992) were used in this study. For DNA in situ hybridization for EBV infection, Not I/ Pst I probe(Montone et al., 1990), a chemically biotin-labeled 23 oligonucleotide from the 125-base pair EBV Not I tandem repeat region which had 91% base homology with a sequence in the Pst I EBV tandem repeat region, was used. And for RNA in situ hybridization, EBERS, EBV encoded mRNAs, probe(Research Genetics Co, USA) was used.

RESULTS

Gross Findings

Gross examination of the jejunum(case 2) revealed a relatively well demarcated large ulcerating mass, 5×4.5 cm, in the wall with diffuse areas of necrosis on cut section(Fig. 2).

Histologic Findings

On light microscopic examination, case 1(Fig. 3) and case 2(Fig. 4) were nearly identical. Both of them are composed of predominantly large atypical lymphoid cells showing vesicular noncleaved and cleaved nuclei with one or two prominent nucleoli and moderate amounts of cytoplasms. There are diffuse scattered tumor necroses. Immunohistochemically, they show uniformly strong positive reaction with leucocyte common antigen. The vast majority of the atypical lymphoid cells showed intense cell membrane staining with L26 (CD20)(Fig. 5) and negative reaction with UCHL-1 (CD45RO) and

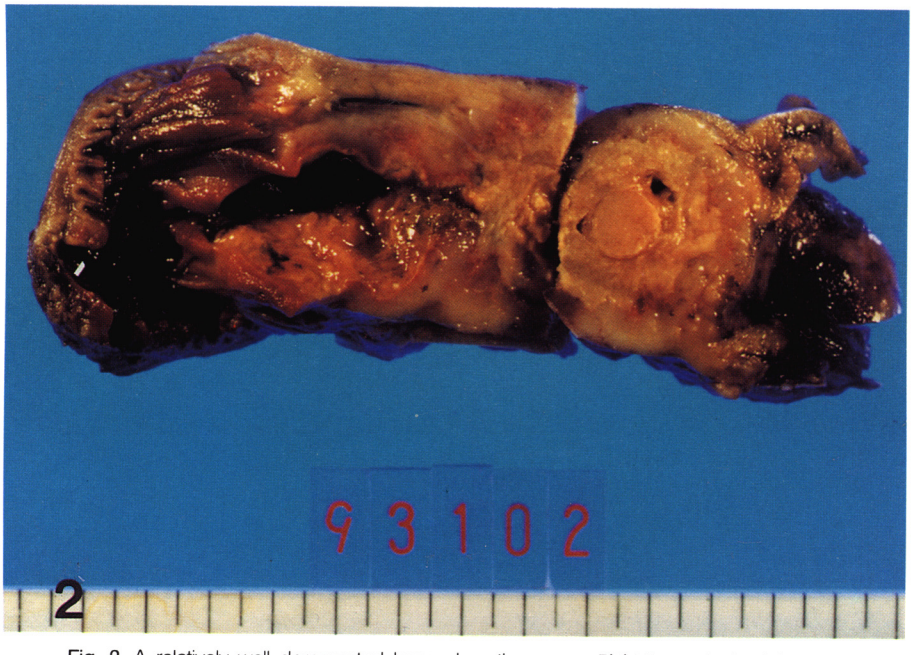

Fig. 2. A relatively well demarcated large ulcerating mass, 5×4.5 cm, in the jejunum.

Table 2. Immunohistochemical staining results

	Case 1	Case 2
LCA	+	+
L26	+	+
UCHL-1	−	−
Cytokeratin	−	−

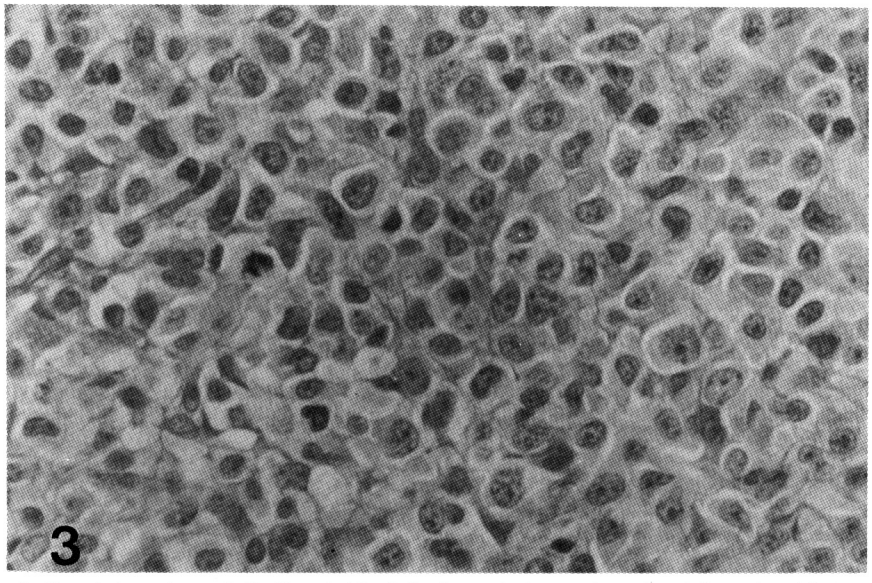

Fig. 3. Case 1 shows large atypical lymphoid cells having vesicular noncleaved and cleaved nuclei with one or two prominent nucleoli.

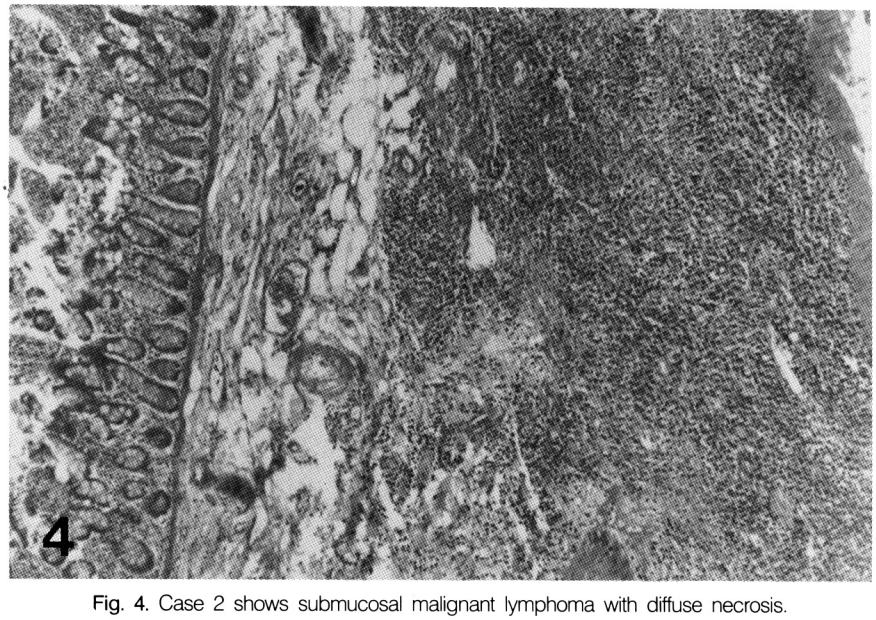

Fig. 4. Case 2 shows submucosal malignant lymphoma with diffuse necrosis.

cytokeratin(Table 2). From histopathologic and immunohistochemical findings, they were considered to be intermediate-grade diffuse malignant lymphoma of the large cell type.

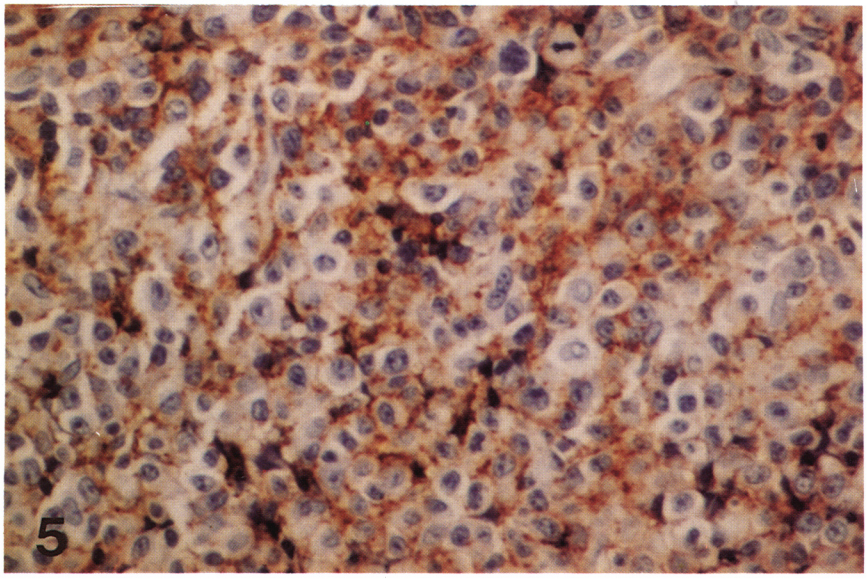

Fig. 5. Immunohistochemical staining with L26 reveals strong positive reaction in the cell membranes.

In Situ Hybridization

DNA in situ hybridization for EBV using NOT I/PST I probe revealed positive signals in a few nuclei in both case 1 and case 2(Fig. 6). RNA in situ hybridization for EBV using EBERS probe showed strong signals in most nuclei in both cases (Fig. 7)(Table 3).

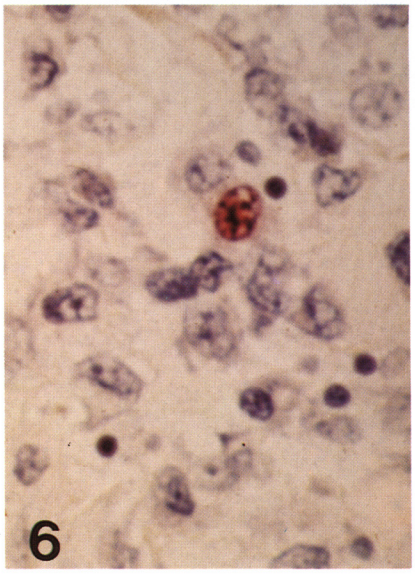

Fig. 6. DNA in situ hybridization for EBV using Not I/Pst I probe reveals positive signal in a few nuclei.

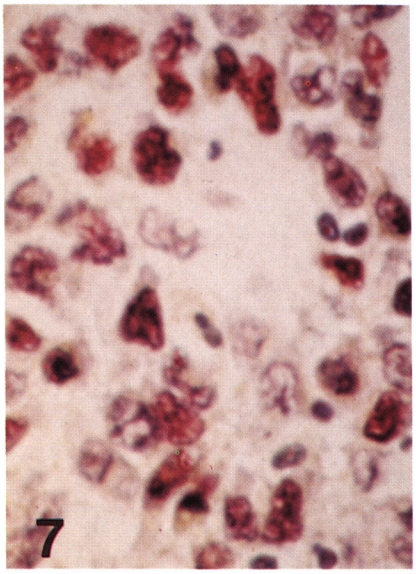

Fig. 7. RNA in situ hybridization for EBV using EBERS probe reveals strong signal in most nuclei.

Table 3. In situ hybridization results for Epstein-Barr virus

	Case 1	Case 2
DNA probe (Not I/Pst I)	+, focal	+, focal
RNA probe (EBERS)	+++	+++

Intensity scale range : 0 ~ ++++

DISCUSSION

It is well known that lymphoproliferative disorders such as diffuse B-cell hyperplasia or B-cell lymphoma are commonly encountered in allograft recipients. Recently, EBV has been implicated in the pathogenesis of these lymphoproliferative diseases occuring in the immunosuppressed recipients of organ transplants(Hanto et al., 1982 &1983 ; Nalesnik et al., 1988 ; Starzl et al., 1984). EBV is a transforming herpesvirus that selectively infects B-lymphocytes, inducing uncontrolled proliferation and mutation which result in malignant transformation. Hanto et al.(1983) have reported 19 cases of B-cell lymphoproliferative disease including 14 cases of B-cell lymphoma and 5 cases of B-cell hyperplasia. And they demonstrated the presence of EBV-specific DNA sequences in biopsy specimens from 12 of the 19 patients by EBV cRNA/DNA filter hybridization, vDNA/DNA reassociation kinetic analysis and Southern blot analysis. In our two cases of malignant lymphoma, we demonstrated the presence of DNA and RNA sequences of EBV in the tumor cells by in situ hybridization.

PTML may be single or multiple, and arises in the central nervous system, the gut, skin and bone other than in the lymph nodes in contrast to non-Hodgkin's lymphoma. In 17 reported cases by Starzl et al.(1984) and the 43 cases of Nalesnik et al.(1988), cadavaric kidney, liver, heart and heart/lung homografts were the sites of involvement. Relative incidental frequency of lymphoproliferative disease was 1.0% for the kidneys, 2.2% for the liver, 1.8% for the heart and 4.6% for heart/lung transplants(Nalesnik et al., 1988). In our two cases, one developed in the submandibular area and the other in the jejunum.

Almost all these tumors are derived from B-lymphocytes, but a few are of T-lymphocyte origin and rarely the graft may be the initial site of the lesion. There has been an autopsy case of peripheral T cell lymphoma of the large cell type reported in a cadaveric renal transplant in a 56-year-old patient(Ulrich et al., 1989).

The time interval between transplantation and tumor detection varies from 0.7 months to 3 years. In Nalesnik's study(1988) 43 cases of lymphoproliferative disorders revealed that the median time of onset in patients initially immunosuppressed with cyclosporin A was 4.4 months after organ transplantation. In our cases, tumors were detected 5 months after KT in both cases. There are several reports that reduction or discontinuation of immunosuppression cause regression of the lesion in most patients(Hjelle et al., 1989 ; Nalesnik et al., 1988 ; Starzl et al., 1984).

Malignant lymphoma in the organ transplant is almost always of host origin but the cases of donor origin has also been rarely reported(Hjelle et al., 1989). These data support the idea that donor cells can undergo malignant transformation in transplant recipients.

In this study, we confirmed that EBV is involved in the development and pathogenesis of posttransplant malignant lymphoma in immunosuppressed patients after kidney transplantation.

REFERENCES

Hanto DW, Frizzera G, Gajl-peczalska KJ, Sakamoto K, Purtilo DT, Balfour HH, Simmons RL, Najarian JS : *Epstein-Barr virus induced B-cell lymphoma after renal transplantation. N Engl J Med 306 : 913-918, 1982.*

Hanto DW, Gajl-peczalska KJ, Frizzera G, Arthur DC, Balfour HH, McClain K, Simmons RL, Najarian JS : *Epstein-Barr virus induced polyclonal and monoclonal B-cell lymphoproliferative diseases occuring after trasplantation. Ann Surg 198 : 356-369, 1983.*

Hjelle B, Evans-holm M, Benedict Yen TS, Garovoy M, Guis M, Edman JC : *A poorly differentiated lymphoma of donor origin in a renal allograft recipient. Transplantation 47 : 945-948, 1989.*

Lezzoni JC, Kang JH, Monton KT, Reed JA, Brigati DJ : *Colorimetric detection of herpes simplex virus by DNA in situ sandwich hybridization. Nucleic Acids Research 20 : 1149-1150, 1992.*

Montone KT, Budgeon LR, Brigati DJ : *Detection of Epstein-Barr virus genomes by in situ DNA hybridization with a terminally biotin-labeled synthetic oligonuc-*

leotide probe from the EBV Not I and Pst I tandem repeat regions. Modern pathology 3 : 89-96, 1990.

Nalesnik MA, Jaffe R, Starzl TE, Demetris AJ, Porter K, Burnham JA, Makowka L, Ho M, Locker J : *The pathology of posttransplant lymphoproliferative disorders occuring in the setting of cyclosporin A-prednisone immunosuppression. Am J Pathol 133 : 173-192, 1988.*

Starzl TE, Nalesnik MA, Porter KA, Ho M, Iwatsuki S, Griffith BP, Rosenthal JT, Hakala TR, Shaw BW, Hardesty RL, Atchison RW, Jaffe R : *Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. Lancet 1 : 583-587, 1984.*

Ulrich W, Chott A, Watschinger B, Reiter C, Kovarik J, Radaszkiewicz T : *Primary peripheral T cell lymphoma in a kidney transplant under immunosuppression with cyclosporin A. Hum Pathol 20 : 1027-1030, 1989.*