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Case Report

The efficacy of EBER in situ hybridization (ISH) stain in PTLD (malignant diffuse large B-cell lymphoma) about 4 years after ABO-incompatible kidney transplantation: a case report

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Abstract: Post-transplant lymphoproliferative disease (PTLD) is a well-known late complication of organ transplantation which incidence has increased after the introduction of more powerful immunosuppressive agents. A 58-year-old man performed ABO-incompatible living kidney transplantation in June, 2008. At 3 years and 9 months after the transplantation, the patient complained of general fatigue and dyspnea and was hospitalized with renal dysfunction. The clinical data in hospital showed acute rejection, but soluble IL-II exceeded 21700U/ml, and HE staining kidney graft showed a massive infiltration of atypical lymphocytes. Atypical lymphocytes were positive for L-26 and negative for CD3 immunochemical stein, and the EBER in situ hybridization stain for EBV was negative in renal graft. We diagnosed diffuse large B-cell lymphoma in the kidney graft. However, he died due to multiple organ failure (MOF). We described a fatal case of diffuse large B-cell lymphoma without EBV infection occurring 3 years 9 months after ABO-incompatible kidney transplantation. Unfortunately, post-mortem autopsy using EBER-ISH stain does not show whether EB virus infection was a cause.

Keywords: PTLD, ABO-incompatible living kidney transplantation, EBER in situ hybridization stain

Introduction

The incidence of malignant tumors after kidney transplantation in Japan is reportedly 5.3-6.8% [1-3],and 8.4-16.7% of the patients who develop malignant carcinoma after kidney transplantation also develop post-transplant lymphoproliferative disease (PTLD) [1, 3]. This rate is lower than that reported abroad. The occurrence of PTLD is associated with the collapse of T-cell-dependent host defense mechanisms under immunosuppressive therapy. Reportedly, the risk factors are aggressive immunosuppressive therapy and infection with viruses such as Epstein-Barr virus (EBV) [4]. Generally, therapies consist in a reduction in the number and/or dose of the immunosuppressive agents or in therapy using anti-cancer agents and rituximab.

Case report

The patient, a 58-year-old man, underwent ABO-incompatible living kidney transplantation in June, 2008. As a pre-operative desensitization therapy, he was administered tacrolimus, mycophenolate mofetil (MMF) and prednisolone. Rituximab 100 mg/m^2 was also given on Day 14 and Day 2 before the transplant. Basiliximab, an anti-CD25 agent, was administered for post-operative desensitization. Pre-operatively, plasma-exchange (PE) was performed three times to remove anti-A antigen IgM and IgG. The kidney began functioning immediately after completion of the transplantation. To relieve the severe diarrhea caused by MMF, the drug was converted to mizoribine on post-operative day (POD) 16. Cytomegalovirus (CMV) infection oc-

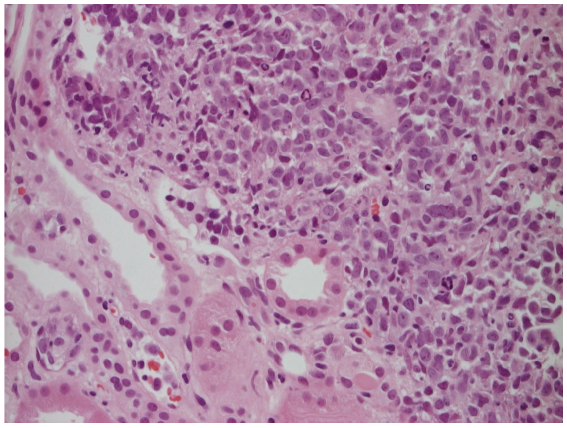

Figure 1. Renal graft showed a massive infiltration of atypical lymphocytes (HE stein).

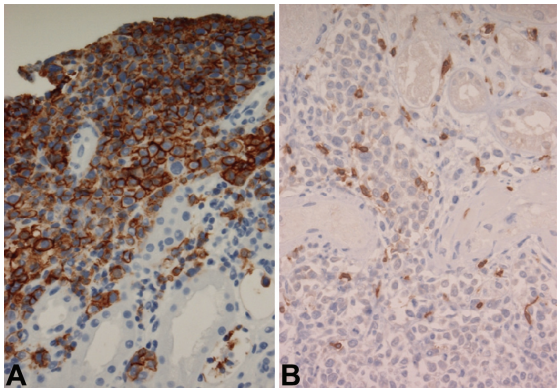

Figure 2. A. Atypical lymphocytes were positive for L-26 in immunochemical stein. B. Atypical lymphocytes were negative for CD3 in immunochemical stein.

curred on POD 32, but it was successfully treated with ganciclovir (5mg/kg, twice daily, for 7 days). A liver biopsy was made to check for liver dysfunction on POD122, because he had been admitted with unexplained liver dysfunction before the operation. The result was mild chronic inactive hepatitis, not a drug-induced condition or viral infection . Acute rejection (AR) occurred on POD 474, but methylprednisolone pulse therapy (250mg/day x 3 days) proved effective and good renal function was maintained. Three years nine months after the surgery, the patient complained of general fatigue and dyspnea; he was hospitalized with deteriorating renal function. On the day of hospitalization, a biopsy of the kidney graft showed AR. Steroid pulse therapy consisting of methylprednisolone 250 mg/day was provided for three days. However, his systemic condition did not improve and his respiratory condition worsened. The histopathological findings that were available four days after his hospitalization were consistent with the diagnosis of diffuse large B-cell lymphoma, because HE staining of the renal graft showed massive infiltration of atypical lymphocytes (**Figure 1**). Six days after hospitalization, the patient suffered multiple organ failure and died. Atypical lymphocytes were immunohistochemically positive for L-26 (**Figure 2A**), and negative for CD3 (**Figure 2B**).The renal graft was negative for Epstein-Barr virus (EBV) assessed by EBER in situ hybridization (ISH) stain (**Figure 3**). EBV antibodies, examined after hospitalization, were all negative, while soluble interleukin-2 (s-IL2) exceeded 21,700 U/mL, a value that indicated acute exacerbation of diffuse large B-cell lymphoma not associated with EBV. The post-mortem autopsy revealed diffuse large B-cell lymphoma in the liver and spleen.

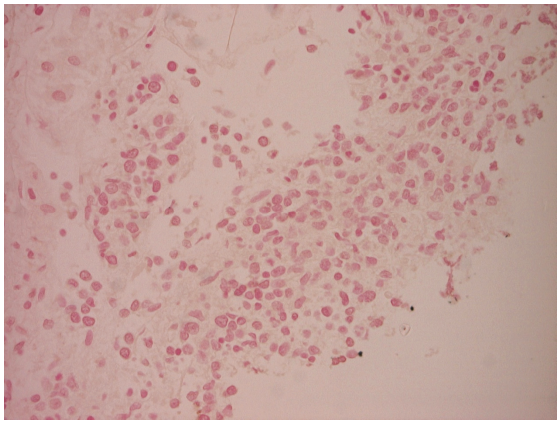

Figure 3. The EBER in situ hybridization (EBER-ISH) stain for EBV was negative.

Discussion

Posttransplant lymphoproliferative disorder disease is due to impaired T-cell immunity. It is a predominantly B-cell malignancy associated with EBV infection in 80-90% of patients with PTLD [5, 6]. I'm afraid we cannot judge whether EBV was the cause of our case, because the EBV antibody test does not enforce for preoperative examination. However, after the examination on admission proved negative for EBV, PTLD was also confirmed to be unrelated to EBV infection by EBER -ISH stain of the graft biopsy specimen. One EBER -ISH method to detect EBV

in pathological tissues uses LMP1 and EBNA2 antibodies. However, some cells infected with EBV do not express LMP1 and EBNA2, because EBER is abundantly expressed as RNA in EBV-infected cells has been reported to be good most sensitive ISH method [7]. This ISH method also serves to distinguish between cell invasion due to AR and cell invasion in PTLD due to EBV infection [4]. Pathological diagnosis of PTLD, which is sometimes T cells and NK cell-mediated, develop abnormal growth of B cells. L -26 staining considers CD20monoclonal antibody as the most reliable B-cell marker and CD3 staining is polyclonal antibody as a most reliable T-cell marker.

We could accurately diagnose diffuse large B -cell lymphoma using these pathological immunochemical methods in this case.

Végső et al reported that strong immunosuppression and calcineurin inhibitors (CNI), EBV-seronegative recipient, EBV-infection, cytomegalovirus infection, human T-cell leukemia virus, AR episode, etc, were risk factor of PTLD [8]. Lowering the risk of PTLD may be achieved by low-dose maintenance immunosuppressive therapy, immunosuppressive drugs that inhibit cell proliferation, and special immunotherapy. Interestingly, Abe et al. [9] reported that none of their 120 renal transplantation patients operated on when conventional therapy with azathioprine and prednisolone was used developed PTLD. All cases of PTLD they had encountered had occurred in patients who had undergone renal transplantation after CNI were introduced in the 1980s. Our patient received an ABO-incompatible kidney transplantation from a living donor, with initial desensitization induced by rituximab therapy and tacrolimus based immunosuppressive therapy for four weeks. In addition, he had experienced an AR episode and CMV infection. We assume over immunosuppression, desensitization and other medications triggered the onset of PTLD after transplantation.

In general, therapy for PTLD includes a dose-reduction of immunosuppressive agents and rituximab [10]. In addition, cytotoxic chemotherapy such a CHOP (rituximab + cyclophosphamide, doxorubicin, vincristine and prednisolone with or without rituximab) is performed when a patient dose not respond to dose-reduction of immunosuppressives and rituximab. Unfortunately, our patient died before treatment for PTLD was provided.

Conclusion

We have described a patient who underwent ABO-incompatible kidney transplantation and who developed diffuse large B-cell lymphoma three years nine months after surgery.

During this time, many cases of PTLD were EBV-associated, but PTLD with negative EBV like our facility's case was very rare.

Possible causes of PTLD are the use of tacrolimus, and administration of immunosuppressive drugs for 4 weeks before TPL as desensitization therapy, which aims to suppress humoral immunity caused by rituximab.

In addition, we consider that the risk factors in this case were CMV infection and acute rejection.

Unfortunately, the post-mortem autopsy using EBER-ISH stain does not show whether EB virus infection was a cause.

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