Case Report

EBV-associated nasal-type NK/T-cell lymphoma of the nasal cavity/paranasal sinus in a renal allograft recipient

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Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a severe complication arising in allograft recipients treated with immunosuppressive drugs. The incidence of PTLD has risen markedly in recent years following the introduction of novel potent immunosuppressants [1]. The spectrum of PTLDs varies from polymorphic B-cell hyperplasia to non-Hodgkin’s lymphoma, which includes monomorphic T-cell lymphomas, peripheral T-cell lymphomas, anaplastic large cell lymphomas and natural killer (NK)/T-cell lymphomas. The majority of PTLDs derive from the B-cell lineage; such lymphomas are often associated with active Epstein–Barr virus (EBV) infection. In contrast to B-cell PTLDs, T-cell or NK-cell PTLDs are associated with EBV infection in only a minority of cases.

We describe here an EBV-associated, nasal-type, extranodal NK/T-cell lymphoma that developed in the nasal cavity/paranasal sinus 4 years after live donor renal transplantation. We have retrospectively examined the association of the EBV titres, determined by serological testing, with lymphocyte cell counts, which were measured during routine out-patient examinations.

Case

A 30-year-old man received a living related renal transplant from his mother in November 1999. The recipient was mismatched at two human leukocyte antigen loci from the donor. After kidney transplantation, the patient was treated with a quadruple immunosuppressive regimen. Treatment consisted of anti-lymphocyte globulin (ALG) at 20 mg/kg from day 1 to day 14, and methylprednisolone (mPSL) given intravenously (i.v.) at 10 mg/kg from day 1 to day 3, followed by 0.8 mg/kg/day orally for the remainder of treatment. This regimen was given in combination with 4 mg/kg mizoribine (MZ) orally and tacrolimus (TAC), given i.v. at 0.1 mg/kg from day 1 to day 7 followed by oral administration adjusted to a 0.4–0.5 mg/kg/day serum level. The doses of mPSL and TAC were tapered to 0.1 mg/kg and 0.1–0.2 mg/kg (target trough 5–10 ng/ml), respectively, within the first 6 months. Mycophenolate mofetil (MMF; 40 mg/kg/day) replaced MZ in November 2000 due to a slight deterioration in graft function. We did not observe any evidence of graft rejection or cytomegalovirus (CMV) reactivation after renal transplantation.

Four years after transplantation, the patient presented with a left facial palsy. Three months later, he was admitted to the Oyokyo Kidney Research Institute, Hirosaki Hospital with a low-grade fever (38°C). Physical examination upon admission revealed intraoral aphthae, left tonsillitis and swelling of the left cheek, although we did not observe any lymphadenopathy or hepatosplenomegaly.

Magnetic resonance imaging (MRI) of the head revealed hypertrophy of the mucosa in the left ethmoid and sphenoid sinuses (Figure 1). His leukocyte count was 7100 cells/mm\textsuperscript{3} (2% eosinophils, 0% basophils, 81% neutrophils, 8% lymphocytes and 8% monocytes). Serum levels of C-reactive protein, lactate dehydrogenase and creatinine were 0.4 mg/dl, 468 IU/l and 1.8 mg/dl, respectively.

Epstein-Barr virus (EBV) BV serology revealed titres of EBV viral capsid antigen (VCA)-specific IgG of 640, EBV early antigen (EA)-diffuse or restricted (DR)-specific IgG of 40, and EBV-VCA-specific IgM of <10. The EBV DNA copy number obtained from peripheral blood mononuclear cells was $3.1 \times 10^3$ copies/10\textsuperscript{6} cells by real-time polymerase chain reaction.
The patient’s condition was complicated by a haemodynamically irrelevant pericardial effusion after admission. EBV was also detected within the pericardial effusion fluid. As the titre of EBV-VCA-specific IgA, characteristic of hypopharyngeal tumours, was ×40 and the levels of soluble interleukin-2 (IL-2) receptor (1604 IU/ml) were elevated, we performed a diagnostic biopsy. A biopsy specimen obtained from the left sphenoid sinus exhibited the proliferation of medium sized cells with small cleaved cell-like nuclei, consistent with an angiocentric lymphoma (Figure 2A). We performed histological and immunohistochemical analyses on formalin-fixed, paraffin-embedded sections from the tumour biopsy. Immunohistochemical studies indicated positive reactivity with cytoplasmic CD3, CD5, CD68 and granzyme B-specific antibodies, a weak reaction with anti-CD8 antibodies, and no reactivity for surface CD3, CD4, CD20, CD56, CD79a, latent membrane protein (LMP)-1 and EB virus nuclear antigen 2 (EBNA2) antibodies. The neoplastic nature of the isolated cells was confirmed by the observation of monoclonal episomal EBV within peripheral blood lymphocytes by Southern blot hybridization using an EBV terminal repeat-specific probe (data not shown).

We also performed in situ hybridization using an EBV-encoded small nuclear RNA (EBER)-1 probe. EBER-1 was strongly expressed by the tumour cells (Figure 2D). Staging studies could not identify any other lesions, suggesting the existence of a tumour. From these results, the patient was diagnosed with stage IA nasal-type extranodal NK/T-cell lymphoma.

We discontinued TAC and MMF administration, but continued low dosage mPSL to avoid the risk of graft rejection according to the patient’s instructions. In addition to reducing immunosuppressant doses, we began a course of the antiviral agent ganciclovir. After excision of the lesion, the patient was treated with irradiation (50 Gy), followed by six courses of chemotherapy. Chemotherapy, comprising ifosfamide (1500 mg/day, day 1), etoposide (100 mg/day, days 1–3), carboplatin (250 mg/day, day 1) and dexamethasone (40 mg/day, days 1–3), resulted in complete disappearance of the tumour. The left facial palsy also resolved. The patient remained in complete remission as evidenced by MRI and retained graft function with no manifestations of rejection for 16 months. The EBV DNA copy number, however, remained high (8.3 × 10^3 copies/10^6 cells); the patient retained elevated soluble IL-2 receptor levels (2305 IU/ml).

Using stored serum samples, we retrospectively examined the variation of the titres in EBV-VCA-specific IgG, IgA and IgM and EBV-EA-DR-specific IgG. The relationship between the titres of EBV and the peripheral blood lymphocyte counts from the time of renal transplantation to the onset of PTLD was also accessed. At the time of renal transplant, the titres of EBV-VCA-specific IgG, IgA and IgM and EBV-EA-DR-specific IgG were negative. Five months after transplantation, we noticed a slight increase in the titres of EBV-VCA-specific IgG and IgA and EBV-EA-DR-specific IgG. Lymphocyte counts increased concomitantly during this time period. Since then, serological screening has exhibited a continuous
increase in the titres of EBV-VCA-specific IgM, EBV-VCA-specific IgG and IgA, and EBV-EA-DR-specific IgG, and lymphocyte counts have gradually decreased at the onset of PTLD (Figure 3).

Discussion

The NK-cell lineage typically displays an immunohistochemical profile of surface CD2⁺, surface CD3⁺/CD56⁺, cytoplasmic CD3⁺ and surface CD56⁺ [2]. Due to limitations in the amount of available tissue, we could not examine biopsy samples for CD2 and perforin immunoreactivity. The tumour cells were negative for the B-cell markers CD20 and CD79a. The observation that the neoplastic cells were positive for cytoplasmic, but not surface, CD3 suggests that these lymphoma cells were derived from the NK-, not the T-, cell lineage [3]. Though the tumour cells were negative for the NK/T-cell marker CD56, it was reported by Quintanilla-Martinez et al. that the expression frequency of the NK/T-cell marker CD56 was ~80% [4]. The presence of an extranodal tumour and late-onset PTLD are consistent with previous reports of NK/T-cell PTLDs. Thirteen cases of EBV-associated PTLDs of the T-cell type have been documented in the literature [5]. Only six cases of PTLDs of the NK-cell type have been reported [6].

Putative NK-cell lymphoma/leukaemias are more common in Asians than in Occidentals. EBV infection is also more prevalent in Asians. These facts suggest that EBV may play an important role in the pathogenesis of this type of tumour [2]. More than 80% of B-cell lymphomas and 30% of T-cell and NK/T-cell lymphomas are EBV positive in Japan [7], as was seen in this patient. Although we did not determine which specific peripheral blood lymphocyte subset was reduced, the general decrease in lymphocyte cell counts suggests that EBV-specific cytotoxic T lymphocytes were either absent or insufficient in number to prevent the proliferation of EBV-infected lymphocytes. Conversely, the increase of lymphocyte counts shortly after renal transplantation suggests primary EBV infection.

EBV-associated malignancies are categorized into three groups according to latent gene expression patterns. Although we did not examine EBNA1 in EBV-related tumours, the patient was negative for both EBNA2 and LMP-1, and positive for EBER, suggesting a latency type I infection. Such a latency type I expression pattern is typically found in Burkitt’s and Hodgkin’s lymphomas. Furthermore, EBV serological testing detected increased levels of serum VCA-specific IgA, a marker characteristic of nasopharyngeal tumours. Despite detection of LMP-1 expression at the RNA level, only 58% of tumour cells exhibited positivity with LMP immunostaining due to methylation [8]. As nasal NK/T-cell lymphoma, Hodgkin’s disease and nasopharyngeal carcinomas frequently lack detectable LMP-1 expression in paraffin sections [5], we cannot exclude a latency type II pattern of infection.

Risk factors for the development of PTLDs after renal transplantation include recipient EBV-negative serostatus at transplantation (increased risk of primary EBV infection), EBV donor/recipient status.
mismatch, paediatric patients, use of ALG or OKT3, CMV infection, and excessive immunosuppression [9]. The patient’s TAC trough levels were not significantly elevated (Figure 3); post-transplantation screening was negative for CMV antigenaemia, providing no indication that the patient was at risk of excessive immunosuppression. Although EBV seropositivity is thought to reach 100% in adults in Japan, this patient remained seronegative for EBV at the age of 30.

Unlike the reactivation of latent EBV, EBV primary infection that occurs during immunosuppressive conditions, such as those experienced following renal transplantation, tends to develop into chronic active EBV infection, often progressing to malignancy. Due to the use of potent immunosuppressants, such as MMF, measurement of TAC trough levels and CMV infection status are insufficient for the assessment of excessive immunosuppressive status. In this patient, the increases in lymphocyte counts observed during primary EBV infection were followed by decreases both during chronic active EBV infection and at the onset of PTLDs; it is not clear, however, whether this variation in peripheral blood lymphocyte counts reflects the excessive immunosuppression or EBV infection. If indicative of active infection, measurement of peripheral blood lymphocytes could be an important index of EBV infection that can be performed in routine outpatient examinations. In EBV-negative patients experiencing primary EBV infection following renal transplantation, unlike patients undergoing viral reactivation, EBV infection probably remains active, even with reduced doses of immunosuppression, as the patient has not generated sufficient anti-EBV antibodies by which EBV becomes inactive.

In general, renal grafts should be sacrificed to rejection before compromising the patient’s life to PTLD. In this case, however, mPSL was never completely withdrawn in accordance with the patient’s hope that the transplant could be rescued. The poor prognosis of NK/T-cell PTLD (mortality rate 80%) [10] and the elevated EBV DNA copy number and levels of IL-2 receptor antibodies detected in this patient indicate a risk of systemic recurrence. Thus, further reduction of mPSL or complete withdrawal should be considered.

Conflict of interest statement. None declared.

References

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