EBV-Associated B- and T-Cell Posttransplant Lymphoproliferative Disorders Following Primary EBV Infection in a Kidney Transplant Recipient

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Abstract

Posttransplant lymphoproliferative disorders (PTLDs) usually are of B-cell lineage and associated with Epstein-Barr virus (EBV). PTLDs of T-cell lineage are much less common and infrequently associated with EBV. We report a rare case of a girl in whom B-cell and T-cell PTLDs developed following 2 EBV-negative kidney transplants. Within 2 years of the second transplantation, the originally EBV-negative patient developed both an EBV-associated clonal B-cell PTLD involving lymph nodes and an EBV-positive T-cell PTLD involving bone marrow and liver. These proliferations occurred concurrently with evidence of primary EBV infection and high plasma viral load. The patient eventually died of multiorgan failure 5 years after the initial transplant (3 years after the second transplant). To our knowledge, only 4 cases of both B-cell and T-cell PTLDs have been reported. Only 2 cases have been proven to be monoclonal and EBV-associated, as in this case, the first following kidney transplantation.
Approximately 75% of T-cell PTLDs reported have been EBV-negative, and these lesions have occurred more commonly after kidney transplantation than after transplantation of other solid organs or bone marrow. Like B-cell PTLDs, T-cell PTLDs tend to arise at extranodal sites, but unlike B-cell PTLDs, the interval between transplantation and the development of PTLDs typically is longer, with a median of 15 years.\textsuperscript{4,5} Patients with T-cell PTLDs frequently have a more aggressive clinical course.\textsuperscript{4,5} T-cell PTLDs express pan–T-cell antigens (eg, CD3, CD5) and T-cell receptors \( \alpha \beta \) or \( \gamma \delta \). Most cases carry monoclonal T-cell receptor gene rearrangements.\textsuperscript{1}

We report an unusual case of a girl in whom 2 PTLDs developed, one of B-cell lineage and the other of T-cell lineage, following allogeneic kidney transplantation. Both PTLDs were monoclonal, proven by molecular studies, and were associated with EBV infection.

**Case Report**

The patient was a 4-year-old African American girl who was seen in February 1997 because of end-stage renal disease secondary to postinfectious glomerulonephritis. She received 2 allogeneic kidney transplants at another institution. The initial transplant was performed in the winter of 1998, and the graft failed shortly thereafter. In April 1998, the graft and native kidneys were removed. After 2 years of hemodialysis, the patient underwent right cadaveric kidney transplantation in February 2000 and received a maintenance immunosuppressive regimen of 25 to 55 mg of cyclosporine 3 times a day and 1 to 3 mg of rapamycin per day. The patient and both allograft donors were seronegative for EBV.

In August 2001, the patient had lymphadenopathy, an elevated serum creatinine level, and loss of vision in the right eye. A cervical lymph node biopsy was performed and showed EBV-associated B-cell PTLD. Polymerase chain reaction (PCR) studies performed on peripheral blood demonstrated an EBV viral load of \( 1 \times 10^6 \) transcripts per liter. The patient subsequently underwent biopsies of left axillary lymph node and liver and bone marrow aspiration and biopsy in July 2002. The lymph node showed recurrent EBV-associated B-cell PTLD. The liver and bone marrow specimens showed an EBV-associated T-cell PTLD. At this time, the patient was treated with ganciclovir, CytoGam, and reduction in immunosuppression (cyclosporine 7 mg twice a day, prednisone 5 mg daily), but no lymphoma-specific therapy was administered. During the next 5 months, the patient’s clinical course was complicated by episodes of infection, liver dysfunction, and renal insufficiency. In September 2002, her EBV titer reached \( 4.6 \times 10^{10} \) transcripts per liter. She died in December 2002 of multiorgan failure characterized by respiratory distress, lung infiltrates, renal insufficiency, and abdominal distention and ileus. Autopsy was not performed.

**Materials and Methods**

**Histologic Examination**

H&E-stained sections from lymph node, liver, and bone marrow aspirate clot and biopsy specimens and Wright-Giemsa–stained bone marrow aspirate smears were reviewed.

**Immunophenotypic Analysis**

Immunohistochemical stains were performed using formalin-fixed, paraffin-embedded tissue sections, heat-induced epitope retrieval, an avidin-biotin-peroxidase complex method, and an automated immunostainer (Ventana-Biotech, Tucson, AZ) as previously described.\textsuperscript{6} The antibodies used for these analyses, although each antibody was not used on all specimens, were specific for CD3, CD8, CD20, and T-cell receptor \( \beta \) (TCR\( \beta \), \( \beta F1 \) (DAKO, Carpinteria, CA); CD4 (Novocastra, Newcastle upon Tyne, England); CD5 (Lab Vision, Fremont, CA); CD56 (Monosan, Burlingame, CA); and TIA-1 (Immunotech, Maravilla, France). All slides were run concurrently with positive and negative control slides.

Immunophenotypic analysis by 4-color flow cytometry was performed on bone marrow aspirate material, as described previously.\textsuperscript{7} The panel included antibodies specific for CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD19, CD20, CD56, and CD57 (Becton Dickinson, San Jose, CA).

**In Situ Hybridization**

In situ hybridization analysis for EBV was performed using formalin-fixed, paraffin-embedded tissue sections, a fluorescein-labeled peptide nucleic acid probe specific for EBV-encoded small RNA (EBER), and the DAKO hybridization kit according to the manufacturer’s instructions, with the appropriate positive and negative control samples.
Cytogenetics

Conventional G-band karyotype analysis was performed on metaphase cells from the bone marrow aspirate specimen cultured for 24 and 48 hours, using previously described methods. The results were reported using the International System for Human Cytogenetic Nomenclature.

Assessment of Clonality

DNA was extracted from formalin-fixed, paraffin-embedded tissue of the cervical lymph node, liver, and bone marrow biopsy specimens and analyzed for immunoglobulin heavy chain (IgH) and T-cell receptor γ chain (TCRγ) gene rearrangements using PCR-based methods. For the analysis of the IgH gene, 3 sets of fluorescent-labeled consensus variable region (V) primers, framework regions (FR) I, II, and III, and a mixture of joining (J) region primers were used. For the analysis of the TCRγ gene, a mixture of fluorescent-labeled consensus V primers and J primers were used. Following PCR, capillary electrophoresis and GeneScan (Applied Biosystems, Foster City, CA) analysis were performed as previously described. A segment of the β-globin gene was amplified as an internal control.

Results

The initial cervical lymph node biopsy specimen, obtained in September 2001, showed diffuse effacement of the architecture by a cytologically polymorphous proliferation of small lymphocytes, plasmacytoid lymphocytes, plasma cells, and clusters of immunoblasts. Immunohistochemical stains demonstrated that the immunoblasts were CD20+ and CD3–. The plasma cells expressed polytypic cytoplasmic immunoglobulin light chains. In situ hybridization for EBER demonstrated that approximately 50% of the lymphoid cells were positive. Antigen-receptor gene rearrangement studies revealed a monoclonal IgH gene rearrangement with a strong smear pattern in the background, consistent with a monoclonal B-cell population with a background of polyclonal B cells. The analysis of the TCRγ gene showed a smear pattern consistent with polyclonal T cells; there was no evidence of monoclonal TCRγ gene rearrangements. On the basis of these findings, the diagnosis of B-cell PTLD, polymorphous type, EBV-positive was established. The subsequent axillary lymph node biopsy specimen obtained in July 2002 showed similar morphologic and immunophenotypic features, consistent with recurrent B-cell PTLD, polymorphous type, EBV-positive.

The bone marrow aspirate smears and clot and core biopsy specimens obtained in September 2002 showed numerous small atypical lymphocytes representing approximately 60% of the bone marrow cellularity. In aspirate smears, the lymphocytes were small with a high nuclear/cytoplasmic ratio; some had features of large granular lymphocytes with moderately abundant pale blue cytoplasm and azurophilic cytoplasmic granules. Another subset of lymphocytes were medium-sized with more open chromatin. A 500-cell differential count revealed 6% myelocytes, 2% metamyelocytes, 16% bands and segmented granulocytes, 2% eosinophils, 56% lymphocytes, and 18% normoblasts. In the clot and core biopsy specimens, the lymphocytes had an interstitial distribution. Hemophagocytosis was absent. Immunohistochemical stains performed on the clot section showed that the majority of lymphocytes were CD3+ TCRβ+, and CD56–.
Immunophenotypic analysis of aspirate material performed by flow cytometry showed a predominance of T cells that were positive for CD2, CD3, CD5, CD7, and CD8 and negative for CD4, CD10, CD16, CD56, and CD57. Very few B-cells (CD19+, CD20+) were present. Antigen-receptor gene rearrangement studies revealed 2 dominant monoclonal TCR\(\gamma\) gene rearrangements, 231 and 240 base pairs, both of which used the V\(\gamma\)\(_I\) gene family, as well as 5 much smaller amplified products using the V\(\gamma\)\(_{II}\) and V\(\gamma\)\(_{III}\) gene families and a faint smear pattern, the latter consistent with polyclonal T cells \(\text{Image 3}\) (top). Analysis of the Ig\(H\) gene showed a faint smear pattern consistent with a few polyclonal B cells; there was no evidence of monoclonal Ig\(H\) gene rearrangement. Conventional cytogenetic analysis performed on bone marrow aspirate cells showed a complex karyotype: 82-86,XXXX,–7,–9,–12,–13,–18,–20[cp10]/77–78,XXX,+1,+2,+4, +6,+12,+17,+20[cp2]/67,XXX,–5,7,12,+16,–19,+21[1]/47,XX,–5,–19,+3mar[1]/46,XX[6].

The liver needle biopsy specimen, also obtained in September 2002, was involved by an infiltrate of atypical lymphocytes with a predominantly sinusoidal growth pattern; atypical cells were also seen within most portal tracts \(\text{Image 4A}\). These cells generally were small to medium-sized with irregular nuclear contours, condensed chromatin, inconspicuous nucleoli, and scant cytoplasm \(\text{Image 4B}\). Mitotic figures were infrequent. Immunohistochemical stains showed that the neoplastic cells were positive for CD3, CD8 \(\text{Image 4C}\), TCR\(\beta\), and TIA-1 and negative for CD4, CD5, CD20, and CD56. In situ hybridization for EBER demonstrated that the majority of the atypical lymphocytes were positive \(\text{Image 4D}\). Antigen-receptor gene rearrangement studies revealed monoclonal TCR\(\gamma\) gene rearrangements with 2 prominent rearrangements of the same size and V\(\gamma\)\(_I\) family usage as those seen in the bone marrow specimen (\(\text{Image 3}\), bottom). In addition, other smaller TCR\(\gamma\) gene rearrangements using the V\(\gamma\)\(_{II}\) and V\(\gamma\)\(_{III}\) gene families,
identical in size to those in the bone marrow specimen, were identified. IgH analysis showed a faint smear pattern; there was no evidence of monoclonal IgH gene rearrangements.

**Discussion**

We report a rare case of a young girl, initially seronegative for EBV, who underwent allogeneic kidney transplantation and who subsequently developed primary EBV infection and 2 EBV-associated PTLDs, the first of B-cell lineage and the second of T-cell lineage. Molecular testing for IgH and TCR gene rearrangements clearly demonstrated independent T-cell and B-cell monoclonal processes. Furthermore, the patterns of disease were distinct with nodal involvement by the B-cell PTLD and marrow and liver infiltration by the T-cell PTLD, resembling hepatosplenic T-cell lymphoma. Our case is highly unusual because both the B-cell and T-cell PTLDs were shown to be monoclonal and EBV-associated.

We have noted reports of 4 other cases of concurrent or composite B-cell and T-cell clonal proliferations arising in the posttransplant setting, which are summarized in Table 1. The most similar case was that of a 45-year-old man with cirrhosis in whom an EBV-associated anaplastic T-cell PTLD developed 4.5 years after liver transplantation. After reduction in immunosuppression and the administration of antiviral agents, an EBV-associated B-cell PTLD developed. Both the B-cell

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**Image 4** Core biopsy of the liver specimen. **A**, The neoplastic cells demonstrate a predominantly sinusoidal infiltrate of lymphocytes with lesser portal tract involvement (H&E, ×100). **B**, The atypical cells are small to medium-sized with irregular to folded nuclei, condensed chromatin, inconspicuous nucleoli, and scant cytoplasm (H&E, ×400). **C**, The neoplastic cells are CD8+ (×400). **D**, In situ hybridization for Epstein-Barr virus–encoded small RNA shows strong nuclear staining in neoplastic cells (×400).
and T-cell PTLDs were monoclonal. Another case was that of a 31-year-old man with aplastic anemia in whom PTLD developed 42 days after bone marrow transplantation. Peripheral blood showed clonal proliferations of both B and T cells, and EBV was detected in B cells and a minor population of T cells. This case was distinct in that the short time to development was more characteristic of the pattern seen in marrow transplant recipients than the longer latency seen in solid organ transplant recipients.

The other 2 cases reported were not as well characterized. One was a kidney transplant recipient in whom an EBV-associated B-cell PTLD of the scalp developed, followed by a monoclonal T-cell population in the peripheral blood. However, it was not shown that the B-cell PTLD was monoclonal, nor was EBV assessed in the monoclonal T-cell population. Another was a kidney transplant recipient in whom a cutaneous T-cell PTLD developed, followed by an EBV-associated PTLD with both B- and T-cell components. The immunophenotype and genotype of the EBV-positive cells was not clear, and EBV was not detected in the cutaneous T-cell lymphoma.

It generally is accepted that EBV has a central role in the pathogenesis of most PTLDs, which develop as a result of a complex interaction of multiple factors, including impaired T-cell surveillance; immunosuppressive regimens; interleukins (ILs) such as IL-4, IL-6, and IL-10; chronic antigenic stimulation by the allograft; and the activation of cellular oncogenes. In the case of EBV-positive PTLDs, the EBV-specific cytotoxic T-lymphocyte responses controlling the EBV-infected B cells are blunted owing to immunosuppression, allowing uncontrolled proliferation of EBV-infected lymphocytes. The low incidence of EBV infection in T-cell PTLDs likely is related to the low infectivity of EBV toward nonneoplastic T cells. The infection of the T-cell PTLD tumor cells in this case is suggestive of an unusually high level of uncontrolled EBV replication in the posttransplant period.

The overall risk of PTLDs is related closely to the degree of immunosuppression, the type of allograft, and the recipient’s pretransplantation EBV status. Most solid organ transplant recipients, such as kidney transplant recipients, experience lower levels of immunosuppression and, thus, have a lower risk of PTLDs. In addition, the frequency of PTLDs is higher in children than in adults, likely related to the higher frequency of EBV seronegativity in children (49%) compared with adults (8%). Indeed, EBV seronegative patients of all ages are at 30- to 50-fold greater risk of developing PTLDs, particularly late onset and rapidly progressive PTLDs. This has been attributed to the lack of anti-EBV memory T cells resulting in poor control of primary and reactivation of EBV infections during the posttransplant period and of subsequent EBV challenges. Thus, the EBV seronegativity and young age of this patient put her in a high-risk group for developing PTLD.

The pattern of hepatic and bone marrow infiltration and cytologic features of the T-cell PTLD in this patient raised a differential diagnosis with de novo hepatosplenic T-cell lymphoma. Hepatosplenic T-cell lymphoma represents fewer than 5% of all peripheral T-cell neoplasms. Patients usually are young and often present similarly with hepatosplenomegaly and clinically significant cytopenias, and the disease pursues a rapidly progressive clinical course. Bone marrow involvement is common in patients with hepatosplenic T-cell lymphoma, and the neoplasm commonly is interstitial and sinusoidal, as was seen in this patient. Hepatosplenic T-cell lymphoma can also occur with increased frequency in posttransplant patients, although the occurrence of EBV in those patients suggests that the tumors might be regarded more properly as T-cell PTLD. Cytogenetic analysis for the presence of isochromosome 7q or trisomy 8, common findings in de novo hepatosplenic T-cell lymphoma, might help to separate these 2 entities in the posttransplant setting.
Furthermore, although most cases of hepatosplenic T-cell lymphoma express the γδ TCR, some neoplasms can express the αβ TCR, as was true in this case.18 It is interesting that we noted that although TCRγPCR studies demonstrated identical gene rearrangements in the T-cell PTLD in the marrow and liver samples, CD5 was positive on tumor cells at one site but not the other. The greater sensitivity of detection of flow cytometry, used only on the bone marrow specimen, is one likely explanation. Clonal evolution is another possibility.

Clinical recurrence of PTLDs has been estimated to occur in approximately 5% of all cases.1 In some cases, the recurrence is morphologically and clonally identical to the original PTLD, while in other cases, PTLDs recur in a more aggressive form with different histologic features.1 It is reported that PTLD clonality might change over time or even might differ between anatomic sites at the same time.20 In the case we report, the differences in anatomic site (lymph node vs liver and bone marrow) and lineage (B-cell vs T-cell) suggest that 2 distinct, unrelated PTLDs occurred in this patient. The molecular results, ie, IgH gene rearrangement without TCRγ gene rearrangement in the B-cell PTLD with the converse in the T-cell PTLD, support this interpretation.

We report the case of a young girl who underwent allogeneic kidney transplantation for end-stage renal disease and in whom 2 distinct PTLDs, one of B-cell lineage and the other of T-cell lineage, subsequently developed. Both PTLDs were associated with EBV infection and arose from primary EBV infection during the posttransplant period. Hemophagocytosis that can associate with both EBV infection and T-cell lymphomas21 was not observed. To our knowledge, only 4 cases of both B-cell and T-cell PTLDs have been reported previously, including 2 cases following kidney transplantation.11-14 Furthermore, this is the first case of PTLD following kidney transplantation in which both the B- and T-cell PTLDs were infected by EBV.

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References


