Detection of Epstein-Barr Virus in Posttransplantation T Cell Lymphoma in a Kidney Transplant Recipient: Case Report and Review

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Epstein-Barr virus (EBV) causes posttransplantation lymphoproliferative disorders (PTLDs) in the setting of immunosuppression. Type of organ transplant, kind and duration of immunosuppressive therapy, age of the patient, and primary EBV infection play a role in the development of PTLDs. Usually, PTLDs are of B cell type and have been strongly associated with active EBV infection. In contrast, posttransplantation T cell lymphomas (PTTLs) are rather unusual [1, 2], and the association of EBV infection with PTTLs has not yet been clearly defined in the literature. In some cases, the diagnosis of PTTL relied only on anatomicopathological features, whereas in others, in which biopsy specimens were tested for the presence of EBV, either there was no biopsy material left or the results were negative [2, 3]. Although the prognosis of PTTLs is poor [4], decrease of immunosuppressive therapy, therapy with antiviral agents, and treatment with monoclonal and polyclonal antibodies can be successful, provided that they are either done or given early in the course of the illness. Therefore, it is necessary to improve the diagnosis PTTL and to assess the precise involvement of EBV in posttransplantation lymphoproliferative disorders.

Case Report

In July 1991, a 47-year-old man with terminal end-stage renal disease received a cadaveric kidney transplant with a negative crossmatch and 4 mismatches (A, B, DR, and DR). He was EBV-seropositive before transplantation; the kidney was from an EBV-seropositive donor. Immunosuppressive therapy consisted of prednisone, cyclosporin A, and induction with antithymocyte globulin. On day 7, a diagnosis of acute rejection was confirmed, and he received treatment with methylprednisolone (700 mg). On day 8, he was febrile, and a chest radiogram showed an alveolar bilateral pattern. Methylprednisolone therapy was discontinued, the dose of cyclosporin A was increased, and azathioprine treatment was introduced. The outcome for the patient was good.

One month later, he was readmitted to the hospital because of fever (temperature, 40°C) without a clinical origin. EBV serology showed evidence of reactivation. In December 1996, he complained of acute abdominal pain, and MRI demonstrated pneumoperitoneum. Laparotomy revealed jejunal perforation of the involved area, and terminal anastomosis was performed. Pathological examination of a jejunal specimen showed ulcerated angiocentric intestinal non-Hodgkin’s lymphoma of T cell origin (CD45RO- and CD3-positive; CD20- and CD79a-negative). There was involvement of the intestinal wall and wide infiltration of the mesentery; regional lymphatic ganglia were not affected. The margins of the resected area were not infiltrated. Immunohistochemical staining for EBV latent membrane protein 1 was positive, as was in situ hybridization with EBV-encoded RNA probes; PCR analysis of biopsy samples revealed EBV DNA sequences (figure 1). All procedures to determine the stage of T cell lymphoma (thoracic CT scanning and bone marrow biopsy) were negative.

Six cycles of chemotherapy with cyclophosphamide, doxorubicin hydrochloride, vincristine, and prednisone were given, and re-evaluation of the patient was negative. Azathioprine therapy was discontinued, and cyclosporin A treatment was continued. As of September 1999, the patient’s condition was good, and there was no evidence of neoplasm.
Figure 1. Agarose gel (2%) electrophoresis of PCR products from amplification of Epstein-Barr virus (EBV) gp220. Lane M, molecular weight marker (100–1000 bp; Gensura, Del Mar, CA); lane 1, negative control (sterile distilled water); lane 2, positive control (DNA extracted from a paraffin-embedded tissue specimen from a patient with Hodgkin’s lymphoma); lane 3, paraffin-embedded surgical sample from the kidney transplant in our patient with T cell lymphoma. Lanes 2 and 3 show the specific 239-bp amplification product of EBV gp220.

Methods

Paraffin-embedded kidney transplant sections from our patient were stained for demonstration of CD45RO, CD20, and CD79a (Dako, Glostrup, Denmark) and CD3 (with use of antibodies from Novocastra Laboratories, Newcastle upon Tyne, United Kingdom). In situ hybridization for EBV-encoded RNA and immunohistochemical detection of EBV latent membrane protein 1 were performed according to instructions of the manufacturer (Dako). EBV detection in DNA extracted from paraffin-embedded tissue was performed by PCR analysis [5]. Positive and negative controls were included in each run. PCR products were analyzed by agarose gel electrophoresis.

Discussion

PTLDs are a severe but rare complication in transplant recipients [4, 6]. At our hospital, only 3 cases of PTLDs have been diagnosed for 250 kidney transplant recipients: the case of T cell lymphoma reported here, a case of B cell lymphoma, and a case of multiple myeloma. After reviewing the literature via MEDLINE, we found only 8 other cases of PTTL in which EBV was demonstrated by 1 or 2 specific methods [1, 4, 6–9]. The clinicopathologic features of the 8 previously described patients are shown in table 1.

The association of PTTL with EBV is still an issue [2–4]. There are several methods for assessing EBV in tissue samples: in situ hybridization with probes directed against EBV-encoded RNA, Southern blotting and hybridization with radioactive probes, immunohistochemical staining for EBV latent membrane protein 1 or EBV nuclear antigen, and detection of EBV-specific DNA sequences by PCR analysis. In the 8 cases of EBV-associated PTTL that were reviewed, EBV was detected by in situ hybridization with EBV-encoded RNA probes. In situ hybridization has been considered the gold standard for detecting EBV in tissue samples because of its sensitivity, specificity, and quickness [4]. As a result, the fact that EBV was not detected in biopsy specimens by this technique led some investigators to conclude that the cases were not associated with EBV, especially when this method was used alone [2]. Recently, some investigators have pointed out that the absence of EBV-encoded RNA detected by in situ hybridization does not always imply the genuine absence of expression of EBV-encoded RNA. Southern blotting has good sensitivity and specificity, but it requires large amounts of DNA and is time-consuming and cumbersome, making it unsuitable for examining a large number of samples.

Although highly specific, immunohistochemical staining for EBV latent membrane protein 1 can be negative for tumors that express only EBV nuclear antigen 1 [4]. Recently, PCR analysis has been used for detecting EBV DNA sequences in frozen or paraffin-embedded tissue samples [5]. It can amplify

Table 1. Clinicopathologic features of previously reported cases of Epstein-Barr–associated posttransplantation T cell lymphoma in 8 men.

<table>
<thead>
<tr>
<th>Case [reference]</th>
<th>Transplant</th>
<th>Patient’s age at diagnosis, y</th>
<th>Time from TR to diagnosis</th>
<th>Tumor site</th>
<th>Clinical outcome</th>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>ISH-EBV</td>
<td>PCR</td>
</tr>
<tr>
<td>2 [6]</td>
<td>Kidney</td>
<td>42</td>
<td>10 mo</td>
<td>Maxillary sinus</td>
<td>Died</td>
<td>+</td>
</tr>
<tr>
<td>3 [8]</td>
<td>Kidney</td>
<td>47</td>
<td>7 y</td>
<td>Celiac lymph nodes</td>
<td>Alive</td>
<td>+</td>
</tr>
<tr>
<td>4 [6]</td>
<td>Heart</td>
<td>58</td>
<td>2 y</td>
<td>Lung</td>
<td>Alive</td>
<td>+</td>
</tr>
<tr>
<td>5 [6]</td>
<td>Heart</td>
<td>11</td>
<td>7 y</td>
<td>Spleen</td>
<td>Died</td>
<td>+</td>
</tr>
<tr>
<td>6 [1]</td>
<td>Liver</td>
<td>46</td>
<td>9 y</td>
<td>Ileum</td>
<td>Died</td>
<td>+</td>
</tr>
<tr>
<td>7 [4]</td>
<td>Heart</td>
<td>15</td>
<td>4 mo</td>
<td>Blood</td>
<td>Died</td>
<td>+</td>
</tr>
<tr>
<td>8 [9]</td>
<td>Kidney</td>
<td>37</td>
<td>1 mo</td>
<td>Kidney</td>
<td>Died</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTES: IHC, immunohistochemical; ISH-EBV, in situ hybridization with EBV-encoded RNA probes; LMP-1, latent membrane protein 1; ND, not done; TR, transplantation; +, positive.

a EBV DNA was detected by means of a 32P-labeled EBV probe.
b ISH was positive only for a few tumor cells; Borisch et al. [7] suggested no pathogenic role for EBV in this case.
small amounts of DNA successfully, provides results quickly, and allows analysis of a large number of samples. Nevertheless, DNA extraction from paraffin-embedded tissue samples involves a great deal of manipulation, and because of its exquisite sensitivity, precautions must be taken to avoid contamination, since it is necessary to include controls in each run [5]. In addition, amplification of paraffin-embedded samples can be hampered by inhibitory substances.

In our case, EBV was detected by immunohistochemical staining, in situ hybridization, and PCR analysis. To our knowledge, only van Gorp et al. [3] have used the combination of three methods like we did to detect EBV in PTLDs [3]. Because it is possible to obtain a false-negative result for EBV by any of the former methods, we believe that it is advisable to combine in situ hybridization with another method, such as PCR analysis, to improve EBV detection in biopsy samples or at least whenever results of in situ hybridization are negative or dubious. By combining techniques, the precise involvement of EBV in PTLDs could be more accurately assessed.

Treatment of PTLDs (such as decrease or discontinuation of immunosuppressive therapy, chemotherapy, and treatment with acyclovir, ganciclovir, IFN-α, and antibodies to CD21 and CD24) has been used alone or in combination with different outcomes [1–4, 6–9]. Our patient was treated with surgical removal of the tumor, decrease in immunosuppressive therapy (discontinuation of azathioprine treatment), and chemotherapy with a good result. Although the optimal therapy for PTLDs is not clear, early diagnosis allows prompt treatment and may result in a better outcome for patients with PTLDs.

References