Short communication

Transmission of donor Epstein-Barr virus (EBV) in transplanted organs causes lymphoproliferative disease in EBV-seronegative recipients


1 Viral Pathogenesis Unit, Department of Clinical Sciences, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK, 2 Microbiology and Tumorbiology Centre, Karolinska Institute, Stockholm, Sweden and 3 Research Haematology Unit and The Department of Cardiothoracic Surgery, Heart Science Centre, Harefield Hospital, Middlesex, UK

Epstein-Barr virus (EBV) is associated with post-transplant lymphoproliferative disease (PTLD). To determine whether the donor EBV isolate is transmitted to the recipient via the allograft and causes PTLD, EBV isolates from four cases of PTLD in cadaveric heart and/or lung transplant recipients were compared with the donor isolates by PCR and DNA sequence analysis. Two recipients who were EBV seronegative at transplantation acquired an EBV isolate indistinguishable from that of the donor and developed PTLD. In contrast, in two patients who were seropositive before transplantation, the donor isolate differed from that present in PTLD of the recipient. The results suggest that the acquisition of donor EBV is a risk factor for PTLD development in a previously seronegative transplant recipient.

Post-transplant lymphoproliferative disease (PTLD) is one of the commonest causes of morbidity and mortality in immunosuppressed solid organ transplant recipients after the immediate post-operative period. PTLD occurs in 1–10% of transplanted individuals, is highest in incidence in the first post-transplant year and is more common in children than adults (Ho et al., 1988). Clinically, PTLD may vary from infectious mononucleosis-like illnesses to solid localized tumours and is fatal in about 70% of cases (reviewed by Thomas et al., 1995).

High cumulative dose of immunosuppressive therapy required for the maintenance of the allograft is a risk factor for the development of PTLD, with those receiving heart and/or lung transplants, retransplants or experiencing recurrent episodes of rejection being particularly at risk. The ubiquitous human herpesvirus Epstein–Barr virus (EBV) is aetiologically associated with PTLD; viral DNA and virus-coded antigens are almost invariably detected in the tumour cells. Around 50% of PTLD cases develop after primary EBV infection, and since children are more likely to be seronegative than adults prior to transplant, this probably accounts for the increased incidence of PTLD in children (reviewed by Thomas et al., 1995).

In order to define intervention strategies for PTLD it is important to determine the source of infecting virus. Since most seroconversions leading to PTLD occur shortly after transplant, the donor organ and/or transfused blood are likely routes of virus transmission. To investigate this, we have exploited the fact that there are numerous short repeat DNA sequences in the EBV genome and the numbers of repeats of a given DNA sequence vary between the viruses isolated from different individuals. Thus, PCR amplification of these repeat regions gives rise to products of unique sizes for individual virus isolates (Falk et al., 1995). In order to compare donor–recipient virus isolates, we decided to amplify the 39 bp DNA repeat region within the coding exon of the EBV nuclear antigen (EBNA)-6 gene, which in the prototype virus B95-8 is repeated three times (Baer et al., 1984).

Four transplant recipients with EBV-associated PTLD were studied. All patients were transplanted with cadaveric heart and/or lungs at the Cardiothoracic Unit, Harefield Hospital, Middlesex, UK. All received a cyclosporin/azathioprine immunosuppression regimen following the transplantation. In addition, acyclovir (ACV) (200 mg five times a day) was given for the first 3 months after transplant. Two patients (T2 and T4)
Table 1. Clinical data on patients with EBV-associated PTLD

Abbreviations used: M, male; F, female; PTLD, post-transplant lymphoproliferative disease; CM, cardiomyopathy; CHD, congenital heart disease; CF, cystic fibrosis; -, EBV seronegative; +, EBV seropositive.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex/age (years)</th>
<th>Primary Transplant type</th>
<th>Pre-transplant EBV status</th>
<th>Onset of PTLD (months post-transplant)</th>
<th>Outcome (months post-PTLD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>F 5 CHD</td>
<td>Heart</td>
<td>+</td>
<td>6</td>
<td>Alive (36)</td>
</tr>
<tr>
<td>T2*</td>
<td>M 18 CF</td>
<td>Lungs*</td>
<td>-</td>
<td>3</td>
<td>Dead (2)</td>
</tr>
<tr>
<td>T3</td>
<td>F 20 CF</td>
<td>Heart-lungs</td>
<td>+</td>
<td>2.5</td>
<td>Dead (1.5)</td>
</tr>
<tr>
<td>T4</td>
<td>M 2 CM</td>
<td>Heart</td>
<td>-</td>
<td>3</td>
<td>Dead† (40)</td>
</tr>
</tbody>
</table>

* Patient T2 was transplanted with double lungs which were rejected and a second double lung transplant was performed 1 month after the first. PTLD developed 3 months after the first and 2 months after the second transplant.
† Patient T4 died from chronic graft failure.

received anti-thymocyte globulin for the treatment of graft rejection.

EBV serological status was determined in all transplant recipients and organ donors by testing sera for IgG antibodies to EBV viral capsid antigen by the standard method of indirect immunofluorescence (Henle & Henle, 1966). Tumour biopsy material was stained for EBNA complex using polyclonal human serum and for EBV encoded small RNAs (EBERs) by routine methods (Brooks & Thomas, 1995).

DNA was extracted from donor spleen lymphocytes, PTLD material and, where possible, recipient peripheral blood mononuclear cells (PBMC) by routine methods (Sambrook et al., 1989). To obtain PCR template, 5 ml of mouth wash was concentrated by centrifugation at 24000 g for 2 h at 4 °C (Sixbey et al., 1989), the pellet resuspended in 50 μl of sterile distilled water and treated with 100 μg/ml of Proteinase K (Promega) for 1 h at 37 °C. Samples were boiled for 10 min before use in PCR. DNA (1 μg) and 20 μl of mouth wash were amplified in a 50 μl reaction volume. Each reaction contained 10 mM-Tris–HCl (pH 8.3), 50 mM-KCl, 1 mM-MgCl₂, 0.01 % gelatin, 200 μM of each dATP, dTTP, dGTP, dCTP, 0.1 μM of each primer and 1 unit of Taq polymerase (Promega). The amplification programme consisted of 3 min initial denaturation at 94 °C, and 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 60 s and extension at 72 °C for 90 s, with a final incubation at 72 °C for 10 min. A 2.5 μl volume of the PCR product from the first run was reamplified in a 50 μl reaction volume by another 30 cycles, using the inner or nested primers (nested PCR; annealing temperature 65 °C). The primers used were specific for a 6 bp repeat DNA sequence in the EBNA-6 gene of EBV genome (outer primer pair, 5' ACA CTT GAG TTC CAT GTC GC 3' and 5' TGT AAT CAC TGG CAA AGG GC 3'; inner or nested primer pair, 5' TAT CGC ACG AAG AAC AAC CCC 3' and 5' AGA TGT GGG AAC TGG GAG ACC 3'). A 10 μl volume of nested PCR product was run on a 1.5 % agarose gel and Southern hybridized with 32P-labelled EBNA-6-specific plasmid probe. The presence of amplifiable DNA in samples was determined by control PCR using human β-globin primers described elsewhere (Saiki et al., 1985).

PCR products were directly sequenced by automated DNA sequencing methods (commercially performed by the Department of Molecular Medicine, King’s College School of Medicine and Dentistry, London, UK).

The clinical data on transplant recipients with PTLD are presented in Table 1. PTLD developed between 2.5 and 6 months post-transplant and was diagnosed by routine histology on the biopsy material. All tumours gave positive staining for EBNA complex and for EBERs. Patients T1 and T3 were seropositive for EBV-specific antibodies prior to transplant whereas patients T2 and T4 were EBV seronegative and seroconverted after transplant (Table 1).

Human β-globin PCR showed the presence of amplifiable DNA in all samples (data not shown). PTLD material from patients T1 and T3 (EBV seropositive at transplant) gave PCR products which differed in size from their respective donors indicating that, in both cases, the donor EBV isolate was not present in the tumour tissue and therefore was not responsible for PTLD development (Fig. 1a). In patient T3 the same isolate was detected in peripheral blood and PTLD tissue suggesting that this was the dominant virus isolate in this individual. In contrast, amplification of the EBNA-6 DNA sequence from the PTLD tissue from patients T2 and T4 (seronegative at transplant) gave bands which were indistinguishable in size from that of their respective donors (Fig. 1a, b). In the case of patient T2, who received a lung transplant on two occasions from two separate donors, the EBV DNA band from the PTLD was identical in size to that of the second donor and differed from that of the first (Fig. 1a). Additional
EBV transmission in transplant recipients

Fig. 1. Comparison of EBV isolates from donor-recipient pairs in patients with PTLD. (a) Lane 1, B95-8, EBV positive marmoset cell line as positive control; lane 2, sterile dH2O as negative control; lane 3, spleen cells of the donor of patient T1; lane 4, PTLD tissue from patient T1; lane 5, Ramos, EBV negative Burkitt's lymphoma cell line used as negative control; lane 6, spleen cells from the first donor of patient T2; lane 7, spleen cells from the second donor of patient T2; lane 8, PTLD tissue from patient T2; lane 9, sterile dH2O; lane 10, spleen cells from donor of patient T3; lane 11, tumour tissue from patient T3; lane 12, PBMC from patient T3 at the time of PTLD; lane 13, Ramos cell line. (b), Lane 1, B95-8 cell line; lane 2, sterile dH2O; lane 3, T4 donor spleen cells; lane 4, PTLD tissue from patient T4; lanes 5-7, PBMC from patient T4 at 3 months, 1 year and 2 years after transplant respectively; lane 8, mouth wash of patient T4 at 7 months post-transplant; lane 9, Ramos cell line. M, molecular mass markers. The 418 bp size of the B95-8 PCR product is indicated. The double bands in lanes 6–8 in (b) are assumed to be due to the nested PCR procedure as their size corresponds to that of the amplification products using outer (782) and inner primers (535 bp).

studies on patient T4 showed an identical sized DNA band from peripheral blood at the time of PTLD development, at 1 and 2 years post-transplant and from mouth wash 7 months after transplant (Fig. 1b).

In order to confirm the identity of the PCR products of patients T2 and T4 and their respective donors, direct DNA sequencing was carried out. Patient T2 and the donor were shown to have PCR products of identical 730 bp DNA sequence with the 39 bp sequence repeated 11 times in both cases. Similarly, both patient T4 and the donor had identical 535 bp PCR products with six repeats. This data strongly suggest the transmission of the donor EBV isolate following transplantation to the previously seronegative recipients.
Herpesviruses as a group are well known to reactivated in the immunocompromised host. Transmission of donor EBV to the recipient has been shown in bone marrow transplant recipients and in one case of kidney transplant recipient (Gratama et al., 1988; Van Gelder et al., 1994). In addition, an earlier case report describes the transmission of EBV from a single donor to two recipients in heart/lungs and kidney with resultant PTLD containing the donor virus in both cases (Cen et al., 1991). We have now demonstrated that donor-derived EBV can be transmitted in heart and/or lungs to the organ recipient and give rise to severe or fatal EBV-driven lymphoproliferation.

The exact mechanism by which the donor virus is transmitted via the transplanted organs to the recipient is not known, although it is known that in solid organ transplant recipients, the virus-infected cells in PTLD are of recipient origin (Cen et al., 1991). Thus, the donor virus may either be transmitted in a cell-free state or be released from the donor cells in the recipient. It is possible that a sufficient number of EBV-carrying B cells remain in blood within the allograft and pass into the circulation of the recipient; the virus would then enter the lytic cycle and infect the recipient B cell population. Alternatively, heart and lung tissue might contain foci of EBV-carrying cells which, in the absence of any pre-existing EBV-specific immune control in seronegative transplant recipients, acts as a site of virus replication. In this regard, all four patients studied received ACV for 3 months. This drug inhibits EBV replication (Colby et al., 1980) and might be expected to prevent reactivation in vivo. However, both our seronegative patients (T2 and T4) seroconverted and developed PTLD while on ACV. Thus, prescribing low dose ACV does not inhibit EBV transmission. Additionally, the fact that patient T4 produced the donor EBV isolate in mouth wash 7 months after seroconversion demonstrates that EBV introduced parenterally can relocate to the pharynx, presumably in a B lymphocyte. However, the cell type which supports virus replication in the pharyngeal epithelium has not been identified.

In our study, both seronegative patients acquired EBV isolates indistinguishable from that of the donor following the transplant and this resulted in the development of EBV-associated PTLD, thus suggesting that the acquisition of donor EBV isolate is an important risk factor for PTLD development in previously seronegative transplant recipients. This route of infection could be prevented by matching donor and recipient for EBV status; however, this is not a practical proposition at present. Alternatively, PTLD could perhaps be prevented by the use of an EBV vaccine or by covering the patient with passively transfused autologous EBV-specific cytotoxic T cells during times of intense immunosuppression (Rooney et al., 1995).

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References


