Epstein-Barr Virus Transmission From a Blood Donor to an Organ Transplant Recipient With Recovery of the Same Virus Strain From the Recipient’s Blood and Oropharynx

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A previous study (Savoie et al., Blood 83:2715, 1994) identified eight transplant patients who acquired Epstein-Barr virus (EBV) infection during the peritransplant period. Three of these patients subsequently developed B-cell lymphoproliferative disease within 4 months of transplantation. Among these, there was a 16-year-old liver transplant patient who was negative for EBV at the time of transplant and who received an EBV-negative organ. After transplant, this patient was transfused with 9 U of packed red blood cells. Eight of the donors were EBV-positive and one was EBV-negative. We succeeded in obtaining spontaneous lymphoblastoid cell lines (LCLs) from the blood of three of these donors, one of whom also yielded a cord-blood line established with his throat-wash EBV. Blood from a fourth donor did not yield an LCL, but his throat washing did have transforming activity when inoculated onto cord-blood leukocytes. We initially could establish spontaneous LCLs only from the recipient’s blood. However, a throat-wash sample taken 11 weeks later did show transforming activity. The recipient was shown to have acquired the EBV infection from one of eight EBV-seropositive blood donors. Analysis of fragment length polymorphisms after polymerase chain reaction amplification of the EBV BamHI-K fragment was used to establish strain identity. Western blot analysis for existence of size polymorphisms in three classes of Epstein-Barr nuclear antigens (EBNA-1, EBNA-2, and EBNA-3) confirmed the DNA results. It is noteworthy that the blood donor responsible for transmitting his EBV strain to the recipient had experienced clinical infectious mononucleosis 15 months before donating blood. Our results may, thus, indicate a requirement for leukodepletion of blood destined for immunosuppressed EBV-negative patients. Finally, blood donors with a recent history of infectious mononucleosis should probably be identified so that their blood is not given to EBV-negative transplant patients. © 1996 by The American Society of Hematology.

The Epstein-Barr virus (EBV) is the cause of heterophile-positive infectious mononucleosis.1 Infection with this virus results in a life-long carrier state. By adulthood, over 90% of individuals are infected.2 Interest in EBV stems from its unique property of immortalizing human B lymphocytes in vitro.3,4 In vivo EBV-induced B-cell expansion is also observed in patients during the acute phase of primary infection by the virus5 and in EBV-positive patients with suppressed T-cell immunity.6,7 Transplant patients on immunosuppressive therapy are, therefore, at risk of developing a lymphoid malignancy known as B-cell lymphoproliferative disease (LPD).8,9 According to some studies, transplant patients who acquire EBV infection immediately before or after transplant surgery show an even higher propensity to develop LPD, compared with transplant patients who were already EBV-infected at the time of transplant.10,11 Most importantly, high levels of EBV viremia occurring soon after transplant seem to correlate with an increased tendency to develop LPD.12,13

While the grafted organ is a likely source of EBV infection in transplant recipients,14 transfused blood also constitutes a possible culprit. In fact, EBV transmission through transfused blood has been reported, largely based on serologic data, in patients undergoing open-heart surgery.15,16 and in other transfused patients17 but, to our knowledge, has not been documented in transplant patients before this report. We now describe a case of LPD in a liver transplant patient whose organ donor was EBV-negative. The central aim of this study was to determine whether EBV could be transmitted to this EBV-negative patient via transfused blood. As at least 90% of adult blood donors are EBV-positive, and given the fact that liver transplant patients require multiple transfusions, we hypothesized that EBV transmission via blood was probable in these patients.

METHODS

Case report. The 16-year-old patient in question was one of a recently studied cohort who participated in an investigation that demonstrated that the EBV load in peripheral blood may be predictive of LPD.15 A diagnosis of type I glycogenosis had been made, and the patient underwent liver transplantation with a cadaveric organ on October 27, 1991. She was immediately placed on cyclosporine, immuran, and sulomedrol/prednisone. For 10 days beginning November 5, 1991, OKT3 was added to the above because the patient showed signs of rejection, as judged by liver biopsy and elevated liver transaminases. She was then discharged, but had to be readmitted on November 25, 1991, because of fever and fatigue. A second course of OKT3 was administered from November 27 to December 6, 1991, after which the liver transaminases were found to increase instead of decrease. Her fever persisted, and a liver biopsy performed at that time showed massive infiltration with immunoblasts. Another significant finding showed a high CD8+ count with respect to CD4+ at 3 months posttransplant (Fig 1), which is consistent with the overall clinical profile of herpesvirus infections.18 Meanwhile, the heterophile agglutinins that were reported as nega-
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Data from the Canadian Red Cross Society (Montreal, Canada). After informed consent, the study nurse obtained biologic samples (blood and throat washings) from the blood donors, in accordance with the ethical standards proposed by the Sainte-Justine Hospital Ethics Committee (Montreal, Canada). Furthermore, because EBV is a cell-associated virus, and given the fact that EBV-positive individuals carry EBV-neutralizing antibodies in their serum, only donors of transfused blood containing cellular components (i.e., whole blood, packed red blood cells, and platelet units) were considered important for EBV transmission and were contacted. At the time of transplant, the organ recipient was transfused with nine such units, eight of which were from EBV-seropositive donors, and one was from an EBV-seronegative donor. Before death, the organ donor received only one transfusion, which was also traced and found to belong to an EBV-seronegative blood donor. Seropositivity in the context of the blood donors is defined by an antiviral capsid antigen titer $\geq 10$.

Leukocyte and throat wash cultures. Whole blood (20 mL) was obtained from the recipient as well as from the eight EBV-seropositive blood donors. The heparinized blood was centrifuged on Ficoll-hypaque density gradients, and the mononuclear cell fraction was harvested, washed, and plated at limiting dilution, as previously described. The cultures were fed twice weekly with RPMI-1640 medium supplemented with 10% fetal bovine serum, antibiotics, and 0.5 $\mu$g/mL cyclosporine A (Sandoz, Montreal, Quebec, Canada). Wells containing transformed cell clumps were expanded. The spontaneous LCLs obtained in this fashion were used for the DNA and protein studies described below.

Throat-wash samples in RPMI-1640 were obtained from all eight seropositive blood donors. Saliva and cell scrapings from the recipient’s oropharyngeal mucosa (over the area of the parotid duct opening) were obtained from the recipient, as previously described. This material was frozen and thawed (to disrupt the cells), filtered through a 0.45-$\mu$m syringe filter unit, and used to inoculate cord-blood leukocytes, as previously described. These were then plated at limiting dilution and fed twice weekly as above. Wells showing transformed cell clumps were expanded and used for the DNA and protein studies described below.

Differentiation of viral strains by analysis of DNA and protein polymorphisms. Genomic DNA was obtained from the cultured LCLs by phenol extraction, and a volume equivalent to 75 to 300 ng of each specimen was used for PCR amplification with BamHI-K primers, as previously described. These primers delineate a polymorphic sequence within the IR-3 repeat region of the EBV genome. Briefly, the PCR was performed in 20 mmol/L Tris-HCl pH 8.4; 50 mmol/L KCl; 2.5 mmol/L MgCl2; 0.1% Triton X-100; 0.01% gelatin; 200 $\mu$mol/L each deoxyadenosine triphosphate (dTTP), deoxycytidine triphosphate (dCTP), and deoxythymidine triphosphate (dTTP); 50 $\mu$mol/L deoxyguanosine triphosphate (dGTP); Pharmacia, Baie d’Urfé, Canada); 150 $\mu$mol/L 7-deaza-dGTP (Pharmacia); 100 pmoL/L each primer (manufactured using the phosphoramidite method at the Institut Armand-Frappier, Laval, Canada). After boiling the reaction mixes for 7 minutes, 2.5 U Tag DNA polymerase (Pharmacia) was added to each tube. The PCR was performed in a Perkin-Elmer thermal cycler 480 (Perkin-Elmer, Mississauga, Canada), programmed for a 30-cycle run, with each cycle set at 94°C for 1 minute, 55°C for 2 minutes, 72°C for 3 minutes, and a 7-minute extension at 72°C at the end of the last cycle. The amplified PCR product was precipitated at 4°C in a solution containing 10 mmol/L Tris-HCl (pH 7.6); 1 mmol/L EDTA, 2.5 mol/L ammonium acetate, 10 ng salmon sperm DNA, and 2.5 vol 95% ethanol. The precipitated DNA was then centrifuged, dried, and resuspended in 50 $\mu$L Tris buffer (10 mmol/L Tris-HCl pH 7.6, 1 mmol/L EDTA). A volume containing 10 $\mu$L of the amplified DNA solution was analyzed by agarose gel electrophoresis followed by Southern blotting.
cells were solubilized in sodium dodecyl sulfate sample buffer, and an amount equivalent to 2 \times 10^6 cells was loaded per well of a 7% polyacrylamide gel. After electrophoresis, gels were blotted onto nitrocellulose and stained in 0.2% Ponceau S. The nitrocellulose was then blocked with 1% albumin and incubated with a human polyclonal serum with high reactivity to the Epstein-Barr nuclear antigens (EBNA). The blot was then washed in Tris-buffered saline solution (10 mmol/L Tris-HCl pH 8.0, 150 mmol/L NaCl) containing 0.05% Tween 20 and incubated with alkaline phosphatase-labeled anti-human IgG (Promega, distributed by Fisher Scientific, Montreal, Canada). After a second series of washes, reactive protein bands were revealed by treatment of the blot with a 2:1 mixture of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates (Promega). For the EBNA-3 Western blot, a 6% polyacrylamide gel was used to better define the larger proteins. It was also necessary to overload the wells in order for the EBNA-3 bands to be visible. Staining was performed as described above, except that a small quantity of affinity-purified EBNA-3b and -3c antiserum (obtained from Drs L. Petti and E. Kieff, Harvard Medical School, Boston, MA) was used to spike the polyspecific EBNA antiserum. These affinity-purified antisera were prepared from the same polyclonal serum used for our usual EBNA immunoblots.

RESULTS

Establishment of cell lines using EBV derived from peripheral blood and saliva of recipient and blood donors. Table 1 summarizes our success in establishing LCLs using peripheral blood obtained from the transplant patient in question and from her eight EBV-seropositive blood donors. Immortalization of cord-blood B cells using throat-wash virus obtained from these same individuals is also indicated. Spontaneous LCLs were established from the recipient’s peripheral blood taken December 10, 1991. A throat-wash sample obtained from the recipient on the same day was inoculated onto cord-blood leukocytes, but did not yield an immortalized B-cell line. This throat washing was also negative for EBV DNA by PCR. However, a sample obtained on February 26, 1992, was able to immortalize cord-blood leukocytes.

Table 1 shows that cultured peripheral blood lymphocytes from only three of the eight EBV-seropositive blood donors gave rise to spontaneous LCLs. Two of these eight donors yielded LCLs from cord-blood leukocytes after inoculation with their throat-wash EBV. It is important to note that the efficiency of obtaining spontaneous LCLs from blood is directly related to the number of EBV-infected B lymphocytes present in the blood sample. This also applies to the efficiency of transmission of EBV. Thus, patients whose blood samples did not show immortalization potential in vitro were also less likely to have transmitted their EBV to the recipient.

Comparison of recipient and donor EBV strains by DNA and protein polymorphisms. Comparison of amplified IR-3 sequences shows DNA size identity between recipient (R) and donor (D1) strains of EBV harvested from the peripheral blood and oropharynx of both individuals. Figure 2 shows Southern blots of PCR-amplified DNA from donor and recipient cell lines. The sizes in base pairs (bp) of the amplified bands in the eight lanes starting from the second lane to the left are 815, 815, 815, 815, 840, 790, 700, and 825, respectively.

Figure 3A and B shows Western blots performed with extracts obtained from cell lines established using peripheral blood from the patient and her blood donors, and from cord-blood lymphocytes inoculated with their oropharyngeal EBV. The EBNA-1 and -2 polymorphisms shown in Fig 3A confirm the DNA results, as do the EBNA-3a, -3b, and -3c polymorphisms shown in Fig 3B. While the EBNA-1/EBNA-2 banding profile for donor 3 (D3) is only marginally different from that of the recipient (R) and D1, the EBNA-3 profile clearly establishes that the EBV strain from D3 is distinct from that isolated from D1 and R. Therefore, the
most likely source of EBV infection in the liver transplant patient was a blood transfusion from D1.

DISCUSSION

This work on EBV transmission in transplant patients was performed in the context of a broader study that sought to investigate whether a correlation existed between the burden of EBV-infected cells in the peripheral blood of pediatric transplant patients and the incidence of LPD. To our knowledge, this report constitutes the first documentation of EBV transmission via blood to an immunosuppressed organ transplant patient. The patient in question experienced protracted infectious mononucleosis at 6 weeks posttransplantation. Furthermore, this is the first study where molecular tools were used to document blood transmission of EBV, thus allowing us to identify, using state-of-the-art technology, the particular blood donor whose strain infected the recipient and to eliminate the possibility of natural transmission via casual contact. The first report of EBV infection transmitted by blood transfusion was documented by Gerber et al in 1969 in four of five EBV-seronegative patients undergoing open heart surgery. In another early study, Henle et al reported that 6 of 18 EBV-seronegative patients acquired EBV infection after transfusions after open heart surgery. In both of these studies, serologic techniques were used to establish primary EBV infection. The latter study also showed that those patients with preexisting antibodies may develop a significant increase in anti-EBV titers within the first month after a transfusion. Other case reports of EBV infection after transfusion also appear in the literature.

EBV infection is usually detectable 2 1/2 to 9 weeks after transfusion. Our own published work, using culture,
PCR, and serology, showed that in a cohort of eight EBV-seronegative transplant patients observed at Sainte-Justine Hospital, all eight became infected within 3 months post-transplant.\(^\text{13}\) Seven of the patients received EBV-positive grafts; only one received a liver from an EBV-seronegative donor. This patient became the focus of the present study. The source of infection was subsequently traced to a blood donor who had experienced infectious mononucleosis 15 months before blood donation. Thus, a recent history of infectious mononucleosis may be a legitimate contraindication to blood donation, or at least to the use of such blood for EBV-seronegative transplant patients. Other reports of EBV transmission by pre-illness blood from an individual in the incubation phase of infectious mononucleosis have been described.\(^\text{20,28}\)

Compared with immunocompetent individuals, patients who are immunosuppressed and EBV-negative may experience higher rates of infection.\(^\text{13,19}\) Because of their deficient T-cell function and lack of EBV-primed memory T cells, the latter patients may be at markedly higher risk of developing EBV-induced B-cell lymphoproliferation.\(^\text{12,13,19}\) In fact, in our own study, three of the eight patients who were EBV-negative before transplant developed LPD.\(^\text{13}\) A recent study, however, demonstrated a direct correlation between EBV viremia and LPD, without regard to the serologic status of the host before transplant.\(^\text{14}\)

The results of this investigation strongly suggest that EBV may be transmitted via blood and cellular blood products to organ transplant patients. This may indicate a requirement to leukodeplete blood destined for certain EBV-negative patient groups, especially those who are immunosuppressed. However, before such decision can be made, a large-scale epidemiologic patient-control study must be undertaken to determine whether or not leukodepletion will, indeed, prevent EBV transmission.

On a more fundamental level, this work is important because it clearly illustrates that the EBV infectious cycle can adopt an alternate route. The virus is naturally transmitted via the oral route; ie, virus excreted in the saliva of EBV-positive individuals may infect oropharyngeal cells of a susceptible host.\(^\text{3,13-34}\) There is current controversy concerning the cell type in the oropharynx that can undergo lytic viral replication and act as a reservoir for EBV. One hypothesis proposes that oropharyngeal epithelial cells allow productive replication of EBV, which may then infect surrounding lymphoid cells\(^\text{35,37}\); the latter harbor the EBV genome in a latent state.\(^\text{38}\) More current data from other groups challenge this view, proposing instead that lymphocytes may be the site of EBV lytic infection and reactivation.\(^\text{39,40}\) Our data indicate that the organ recipient was infected via transfused blood. Blood collected 6 weeks after transplant gave rise to an EBV-positive LCL. A throat-wash sample obtained at the same time as this blood sample did not show transforming activity, but one obtained 11 weeks later (ie, 17 weeks after transplant) readily immortalized cord-blood lymphocytes. The viral strains isolated from the peripheral blood and throat washing of one blood donor (D1) were identical—by DNA and protein polymorphism analyses—to the EBV strains isolated from the recipient's blood and throat washing. Our own data support the view that EBV may lytically infect B lymphocytes (or other lymphoid cells), which can transmit the virus after blood transfusion to an EBV-negative recipient. Still, our results do not exclude the possibility that EBV harbored in lymphocytes might infect oropharyngeal epithelial cells, which in turn would lytically replicate the virus.

ACKNOWLEDGMENT

We gratefully acknowledge the assistance provided by the Canadian Red Cross Society (Montreal Center) in contacting the blood donors who participated in this study. We sincerely thank the eight blood donors who cheerfully agreed to provide blood and throat-wash samples. The assistance of Liliane Gallant with word processing is also gratefully acknowledged.

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