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Reconstitution of EBV-Specific T Cell Immunity in Solid Organ Transplant Recipients

Tanzina Haque,² Peter L. Amlôt,† Nabeela Helling,‡ J. Alero Thomas,§ Paul Sweny,‖ Keith Rolles,¶ Andrew K. Burroughs,¶ H. Grant Prentice,‡ and Dorothy H. Crawford*†

EBV-specific autologous CTL were grown in vitro from three adults (two liver transplant recipients and one patient on hemodialysis awaiting kidney retransplant). All CTL lines were TCR αβ, CD8 positive cells, EBV specific, and MHC class I restricted. The CTL lines were expanded in vitro and infused in three escalating doses (5 × 10⁷, 1 × 10⁸, and 2 × 10⁹) at monthly intervals. Weekly blood samples were collected following each infusion. EBV-specific CTL precursor cells in peripheral blood were quantitated by limiting dilution analysis, and their effect on EBV load in vivo was assessed by semiquantitative PCR. In all three patients, the numbers of CTL precursor cells increased during the weeks following the infusions and were highest after the third infusion. This level gradually declined but remained above the preinfusion levels for up to 3 mo. EBV genome copy number, on the other hand, dropped following the first infusion and became undetectable thereafter. The EBV DNA level remained lower than the pretransplant level in all patients for up to 3 mo after the last infusion. Our study shows that it is feasible to generate and expand EBV-specific CTL from pretransplant blood samples of solid organ transplant recipients, that these CTL can be stored and infused posttransplant, and that they remain cytotoxic and EBV specific in vivo. The aim of this study is to use these CTL for prevention and treatment of lymphoproliferative disease in solid organ transplant recipients.


Following primary infection, EBV persists as a low-level, latent infection in circulating B cells and a replicative/permissive infection in the oropharynx (1–3). This persistent infection is kept at subclinical levels in immunocompetent individuals by EBV-specific CTL, which specifically recognize EBV Ags on the surface of the infected cells. These CTL are CD8 positive and MHC class I restricted (4, 5). In transplant recipients, the use of immunosuppressive drugs to prevent graft rejection leads to suppression of CTL function, thereby generating an environment in which EBV-infected B cells can proliferate (6). In this setting, B cell lymphoproliferative disease (BLPD) develops in 1 to 10% of transplant patients and is associated with a high patient mortality (7). First line of treatment in most transplant centers is reduction or complete withdrawal of immunosuppressive therapy, which allows reactivation of EBV-specific CTL, resulting in partial or complete regression of the tumor in many cases (8). However, recurrences occur that exhibit decreased sensitivity to this conservative form of treatment, and cytotoxic drug therapy is then often required (9).

To date no drug-controlled trial has been performed to define an optimal BLPD treatment strategy, but a search for a nontoxic alternative to cytotoxic drug treatment has led to recent attempts at immunotherapy. In one trial, five allogeneic bone marrow transplant recipients with BLPD were treated with infusions of leukocytes from their EBV-seropositive donors. Regression of the tumor was noted in all cases, presumably due to infused CTL specific for EBV-infected targets, but fatal pulmonary complications occurred in two patients and graft-vs-host disease developed in all five (10). In this system, the beneficial contribution of a graft-vs-tumor effect on tumor regression could not be assessed.

A more recent approach to persistent herpes virus infection in the immunocompromised host is adoptive transfer of cellular immunity in the form of specific CTL. This has been pioneered by Riddell et al. (1994), using human cytomegalovirus-specific CD8 positive CTL clones generated in vitro from bone marrow transplant donor leukocytes. When infused into bone marrow recipients, short-term reconstitution of human cytomegalovirus-specific immunity was achieved (11). In a similar study, CD8 positive, EBV-specific CTL lines were generated in vitro from donor blood and used successfully to prevent and treat BLPD in bone marrow transplant recipients (12, 13). In these studies, donor CTL reconstituted EBV-specific immunity for as long as 18 mo.

To date, all EBV-specific CTL therapy has been conducted in bone marrow transplant recipients where blood from the healthy bone marrow donors is available for the generation of CTL. A similar strategy in solid organ transplant recipients, who have a higher incidence of BLPD, but where the donors are generally unavailable, would require recipient CTL to be grown from pretransplant blood samples taken before the start of immunosuppressive therapy. We report a pilot study undertaken to test the feasibility of EBV-specific CTL therapy in solid organ graft recipients. CD8 positive CTL lines generated from pregraft samples of three solid organ (two liver, one kidney) recipients were successfully expanded in vitro and, following transplantation, infused into the

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further 2.5 PBMC were plated at 2 Generation and expansion of EBV-specific CTL and the resulting LCL were expanded to 25 cm² flasks. LCLs were main-
m culture medium from the B95-8 (EBV-positive) cell line (15) and 1 m
Generation of LCL were used as stimulators to grow autologous EBV-specific CTL that were (emulsified cyclosporin) at 75 mg twice daily. None of the patients re-
(FK506) at 2 mg in the morning and 3 mg at night and the other Neoral the time of study, one liver transplant patient was receiving Tacrolymus
discontinued. He has since been maintained on regular hemodialysis. At
no EBV-associated disease, whereas the third (patient 3) had received a
54 years). Two patients had liver transplants (5 and 6 mo previously) and
infused with CTL. These three patients were adult, males (aged 29, 51 and
restimulated with autologous LCL (4:1 ratio) every 7 days. When sufficient

Materials and Methods

Patient Details
Ethical approval for this study was obtained from Ethical Committees at the Royal Free Hospital and the London School of Hygiene and Tropical Medicine, and patients gave informed consent before entry into the study.

CTL lines were grown from six EBV-seropositive adults (age range 29 to 61 years; HLA types shown in Table I), and three were subsequently infected with CTL. Three of the patients were adult, males (aged 29, 51 and 54 years). Two patients had liver transplants (5 and 6 mo previously) and no EBV-associated disease, whereas the third (patient 3) had received a kidney transplant 9 years earlier but underwent a transplant nephrectomy after 3 mo, when the patient developed BLPD and immunosuppression was discontinued. He has since been maintained on regular hemodialysis. At the time of study, one liver transplant patient was receiving Tacrolimus (FK506) at 2 mg in the morning and 3 mg at night and the other Neoral (emulsified cyclosporin) at 75 mg twice daily. None of the patients received any corticosteroids or mycophenolate therapy.

Peripheral blood (50 ml) was taken pretransplant and used to generate autologous EBV-positive B lymphoblastoid cell lines (LCLs). These LCLs were used as stimulators to grow autologous EBV-specific CTL that were then infused into three patients at three escalating doses of 5 × 10⁶, 1 × 10⁷, and 2 × 10⁷ at monthly intervals. Blood samples were taken before and at weekly intervals following each infusion and were assessed for EBV CTL precursor frequency by limiting dilution analysis and for EBV DNA by PCR.

Generation of LCL
PBMC were separated from whole blood by Ficoll-Hypaque density gra-
dient (14). PBMC (1 × 10⁶) were incubated with 100 μl of supernatant cultured medium (RPMI 1640, B95-8 EBV-seropositive cell line (15) and 100 μg/ml of cyclosporin (Sandoz Pharmaceuticals, Surrey, U.K.) for 1 h at 37°C. The cells were washed once, resuspended in tissue culture medium (RPMI 1640 (Life Technologies, Paisley, U.K.) containing 100 IU/ml penicillin, 100 μg/ml streptomycin, 10% v/v FCS (HyClone, Logan, UT)), and seeded in flat-bottom microtiter wells at a concentration of 1 × 10⁶ cells/well in 200 μl tissue culture medium. The cultures were maintained by weekly feeding, and the resulting LCL were expanded to 25 cm² flasks. LCLs were main-
tained in culture medium containing acyclovir (100 μM; Glaxo-Wellcome, Kent, U.K.) to prevent lytic replication. Regular testing by indirect immu-
nofluorescence for viral capsid Ag (VCA) showed no evidence of lytic Ag expression (16).

Generation and expansion of EBV-specific CTL
PBMC were plated at 2 × 10⁶/well in tissue culture medium in 24-well plates with 5 × 10⁶ autologous LCL that had been x-irradiated at 40 Gy to prevent proliferation (40:1 PBMC:LCL ratio; Ref 17). After 10 days the cells were subcultured at a concentration of 1 × 10⁶ cells/well, and a further 2.5 × 10⁶ x-irradiated autologous LCL (T cell:LCL ratio, 4:1) were added. After 14 days 20 U/ml of IL-2 (Eurocetus U.K., Middlesex, U.K.) was added to each well. Cultures were maintained routinely in tissue cul-
ture medium with the addition of 20 U/ml of IL-2 every 2 to 3 days and restimulated with autologous LCL (4:1 ratio) every 7 days. When sufficient numbers of CTL had been obtained (usually after 2 to 3 mo of culture), aliquots were analyzed for cytotoxic function and cell surface phenotyping.

HLA typing and sterility testing were conducted before washing the cells five times in HBBS (Sigma, Dorset, U.K.) containing 10% human serum albumin (BioProducts, Hartordshire, U.K.,), freezing at 1 × 10⁶ cells/vial in autologous serum containing 10% DMSO (tissue culture grade, Sigma), and storing in the vapor phase of liquid nitrogen.

Chromium release assay for EBV-specific cytotoxicity
Each T cell line was tested for cytotoxic activity against the autologous LCL, an HLA-mismatched LCL, and K562, a NK cell-sensitive target (18). Cells (10⁵) from each target cell line were labeled with ¹¹⁹ Chromium (¹⁹ Cr 100 μCi; ICN Pharmaceuticals, Costa Mesa, CA) for 1 h and plated with CTL at effector:target ratios of 40:1, 20:1, 10:1, and 5:1. After 4 h, the release of ¹¹⁹ Cr from lysed cells was measured on a gamma counter (Wall-
alc, Milton Keynes, U.K.). Percent specific lysis was calculated using the for-
ula: (test sample − spontaneous release)/(maximum release − spontaneous release) × 100.

Surface marker analysis
The phenotype of the CTL lines was analyzed by FACS analysis after staining with mAbs against TCR gd, TCR y, CD4, CD8, CD16, CD25, CD26, CD38, CD45RA, CD45RO, CD54, CD57, and monomor-
phic anti-HLA class II DR, DP, and DQ), as described previously (19).

Safety measures taken before the infusion of CTL
Rigorous precautions were taken during the culture period to maintain sterility of the cell lines. The laboratory strain of EBV, B958, used to generate autologous LCL was screened for mycoplasma regularly using a commercially available detection kit (Gen-Probe, San Diego, CA) and for type D retrovirus (Ref 20; tests kindly conducted by Ms. E. Grogan, Yale University Medical School, New Haven, CT). All LCL were grown in medium containing the antiviral agent acyclovir, which inhibits the EBV late lytic cycle and thereby prevents virus production (21). The LCL were constantly negative for EBV late lytic cycle Ag, VCA, by indirect immu-
nofluorescence techniques. Additionally, to prevent viable LCL cells used as stimulators from contaminating the T cell infusions, tests were per-
formed to obtain an optimum dose of irradiation (40 Gy) that was sufficient to prevent their proliferation. To exclude other infectious contaminants, CTL cultures were subjected to a full microbiological screen for bacteria, fungi, and mycoplasma (conducted at University College, London, U.K.) before being stored frozen. Similarly, to exclude the possibility that the cell lines had been contaminated with allogeneic cells during the culture period, HLA typing was conducted before freezing. All CTL lines were found to consist of the respective recipient’s HLA type only (HLA typing was conducted at the Anthony Nolan Trust, The Royal Free Hospital, London, U.K.).

Infusion of CTL
Three escalating doses of CTL (5 × 10⁶, 1 × 10⁷, and 2 × 10⁷) were infused at four weekly intervals. Frozen CTL were thawed at 37°C, washed once in HBSS containing 10% human serum albumin to remove DMSO, resuspended in 20 ml of HBSS and 10% albumin, and infused slowly over 15 min by i.v. injection. Patients’ vital signs were monitored for 4 h to detect any infusion-related toxicity. The patients were infused as outpa-
tients and did not require hospital admission for the procedure.

Limiting dilution analysis (LDA) for EBV-specific CTLp frequency
PBMC were seeded into 96-well, round-bottom microtiter plates in dou-
bling dilutions at concentrations ranging from 20,000 to 625 per well. Twenty four replicate wells were plated at each concentration, and 5000

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>HLA Class I Types</th>
<th>HLA Class II Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>A2, A19</td>
<td>B12, B51, Bw4</td>
</tr>
<tr>
<td>Patient 2</td>
<td>A9, A19</td>
<td>B7, B40, Bw6</td>
</tr>
<tr>
<td>Patient 3</td>
<td>A2, A3</td>
<td>B5, B8</td>
</tr>
<tr>
<td>Patient 4</td>
<td>A11, A19</td>
<td>B12, B22, Bw4, Bw6</td>
</tr>
<tr>
<td>Patient 5</td>
<td>A26, A31</td>
<td>B8, B51</td>
</tr>
<tr>
<td>Patient 6</td>
<td>A2, A32</td>
<td>B7, B27, Bw4, Bw6</td>
</tr>
</tbody>
</table>

* ND, not done.

b Patients not infused with CTL.

Table I. HLA types of the patients

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irradiated (40 Gy) autologous LCL in 200 μl culture medium were added to each well. rIL-2 (5 U/ml) was added on days 3 and 7. The cytotoxic activity was assessed after 10 to 14 days by the standard 4-h chromium release assay. The contents of each well were split into two and tested against 51 Cr-labeled autologous and HLA-mismatched LCL targets. Any release assay. The contents of each well were split into two and tested against 51 Cr-labeled autologous and HLA-mismatched LCL targets. Any

DNA extraction kit (Invitrogen, San Diego, CA). Total DNA was extracted and subjected to DNA extraction kit (Invitrogen, San Diego, CA). DNA extraction kit (Invitrogen, San Diego, CA). All samples were routinely amplified using primers specific for the human β-globin gene as an internal control (25). PCR was conducted using a primer set that amplifies a sequence (116 bp long) of the EBV genome within the EBNA 2 gene (26). PCR products (10 μl) were run on a 2% agarose gel and Southern hybridized using [α-32P]dCTP-labeled EBNA 2-specific plasmid PM BanHi-H2 (27). With every PCR the sensitivity of the EBNA 2 primer set was determined using Namalwa, an EBV-positive Burkitt lymphoma cell line known to carry 1 to 2 integrated EBV genome copies in each patient. Values for each variable were expressed as percentage of positive cells for surface markers by FACS analysis.

**Results**

**Phenotype of in vitro generated and expanded CTL lines**

The immunophenotype of the six CTL lines was determined by FACS analysis using mAbs against different T, B, and NK cell surface Ags (Table II). The CTL lines consisted mainly of T cells expressing TCR αβ surface markers, and, in five of six cases, virtually all cells were cytotoxic cells (CD8 positive) with only a very few remaining CD4-positive cells (range 0.1–7%). The sixth CTL line (patient 6) contained 80% of CD4-positive T cells. Most cells expressed HLA-DR Ag, and a variable proportion expressed the costimulatory molecule, CD28 (16.8–41%; data not shown). No CD19-positive B cells and very few CD16/CD7+ NK cells (0–1.7%) were detected.

**Table II. Immunophenotype of CTL lines**

<table>
<thead>
<tr>
<th>CTL Line</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>TCR αβ</th>
<th>TCR γδ</th>
<th>CD19</th>
<th>CD16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>98.3</td>
<td>0.1</td>
<td>97.9</td>
<td>92.2</td>
<td>0.2</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Patient 2</td>
<td>96.3</td>
<td>4</td>
<td>91.9</td>
<td>96.2</td>
<td>1</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Patient 3</td>
<td>89.9</td>
<td>3.7</td>
<td>82.3</td>
<td>87.7</td>
<td>0.4</td>
<td>0</td>
<td>1.7</td>
</tr>
<tr>
<td>Patient 4</td>
<td>90.1</td>
<td>2.9</td>
<td>91.9</td>
<td>88.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient 5</td>
<td>90.1</td>
<td>6.9</td>
<td>88.2</td>
<td>90.9</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patient 6</td>
<td>97</td>
<td>79.1</td>
<td>1.3</td>
<td>87</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Results are expressed as percentage of positive cells for surface markers by FACS analysis.

**Characteristics of the cytotoxic function of CTL lines**

Each CTL line was tested against three different target LCL, the autologous LCL, an HLA-mismatched LCL, and the NK target, K562, in 4-h standard chromium release assays. All CTL lines showed significant cytotoxic function against EBV-immortalized autologous LCL targets (Table III; range 31–68% cytotoxicity), when compared with EBV-immortalized, HLA-mismatched LCL targets (p < 0.05). To determine whether the killing was MHC class I restricted, autologous LCLs were preincubated for 30 min with a mouse mAb against an HLA class I determinant (W6/32) and then used as an additional target (29). The HLA class I Ab significantly blocked the killing of the autologous LCL (p < 0.05). These results indicate that the CTL were EBV specific and MHC class I restricted. In addition, the CTL line from patient 6 was tested against autologous LCL, preincubated with anti-HLA class II Ab, which showed a reduction in killing (data not shown), thus indicating that the cytotoxic function of that particular CTL line was both MHC class I and class II restricted.

**Infection of autologous CTL**

No adverse effects of the infections were noted and no changes in vital signs were detected (blood pressure, pulse, respiration, or temperature). An aliquot of cells was retained to confirm EBV-specific cytotoxicity after freeze/thawing procedure. There was no graft dysfunction during the period of observation or for the 9 mo afterward.

**Determination of CTLp frequency in peripheral blood following CTL infusions**

Blood samples taken before, 4 h after, and at regular intervals following each infusion were used to determine EBV CTLp frequency by limiting dilution analysis. Patients 1 and 2 had detectable numbers of CTLp in their pretransplant blood samples (15 and 19/106 PBMC respectively), but these were undetectable in peripheral blood before the first CTL infusion, when they were receiving posttransplant immunosuppressive therapy. EBV CTLp were detectable in the blood samples taken 4 h after the first CTL infusion and increased with each subsequent infusion (Fig. 1, A and B). In patient 3, who was on hemodialysis throughout the study period, CTLp were present at low level (25/106 PBMC) before the first infusion (Fig. 1C), and this level increased after each subsequent infusion. The highest numbers of CTLp were detected after the third infusion in all patients; this level gradually declined but remained detectable for 3 mo. The CTLp were EBV specific and MHC restricted. No significant cytotoxicity was noted in wells containing MHC-mismatched target cells.

**Table III. Cytotoxicity of CTL lines against autologous, HLA-mismatched and NK target cell lines**

<table>
<thead>
<tr>
<th>CTL Line</th>
<th>Autologous LCL</th>
<th>Autologous LCL + Anti-HLA Class I Ab</th>
<th>HLA-Mismatched LCL</th>
<th>NK Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>68</td>
<td>6</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Patient 2</td>
<td>31</td>
<td>0</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>Patient 3</td>
<td>32</td>
<td>12</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Patient 4</td>
<td>50</td>
<td>6</td>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>Patient 5</td>
<td>62</td>
<td>14</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Patient 6</td>
<td>74</td>
<td>27</td>
<td>17</td>
<td>10</td>
</tr>
</tbody>
</table>

* Results are shown as percent specific lysis of target cells at an E:T ratio of 20:1.

P < 0.05, compared with autologous LCL.

ND, Not done.

* Patients were not infused with autologous CTL.
The effect of CTL infusions on EBV DNA levels in peripheral blood

The effect of CTL infusions on EBV DNA in PBMC of the patients was determined using semiquantitative PCR (Figs. 1A and 2A; Figs. 1B and 2B). Patient 3 had detectable EBV DNA in the preinfusion sample, which decreased gradually following CTL infusions and became completely undetectable following the last CTL infusion. The EBV DNA was detected 3 mo following the last infusion, albeit at a lower level than before the infusion (Figs. 1C and 2C). A significant, inverse correlation was observed between the numbers of CTLp and EBV genome copies in peripheral blood in two patients (patients 2 and 3) using Spearman’s rank correlation test (two-tailed p values < 0.04 and 0.0125, respectively).

Discussion

This study investigates the feasibility of using cytotoxic T cell infusion as a treatment strategy in solid organ transplant recipients. The study was conducted on EBV-seropositive transplant recipients who did not have EBV-related disease. One patient had previously developed BLPD following a renal transplant and underwent complete long-term remission after withdrawal of immunosuppressive therapy and removal of the graft. At the time of infusion of CTL, this patient was supported on regular hemodialysis and had been free of BLPD for 9 years.

As indicators of the function of the infused CTL, the levels of EBV DNA and CTLp in peripheral blood were monitored. The results show that functional CTLp could be detected immediately after the first infusion and that they remained above preinfusion levels for up to 3 mo after the last CTL infusion. Each infusion increased the CTLp level to above that seen before the infusion.
The CTL infusions produced rapid drop of EBV genome copies in PBMC to undetectable levels in all three patients after the first infusion and the maintenance of a level lower than that seen in the pretransplant blood samples for 3 mo after the last infusion. Taken together, these data suggest that even the lowest dose of CTL used in this study ($5 \times 10^5$), given every 3 to 4 wk, would be sufficient to prevent or treat BLPD. This estimate is similar to those deduced from other studies in bone marrow transplant recipients (12, 13).

In this study, EBV-specific CTL were grown from six patients, and, in three cases, autologously reinfused without deleterious effect. Clinical and biologic testing clearly demonstrates that it is possible to generate EBV-specific CTL from pretransplant recipient blood samples, that these can be grown up to high cell numbers in vitro, and that they can be stored frozen without loss of function. Five of the six CTL lines generated (including the three that were infused) showed a CD8-positive, activated T cell phenotype, with less than 2% contaminating CD16-positive NK cells and no detectable CD19-positive B cells. One (patient 6) of the six T cell lines generated contained 80% CD4-positive T cells (CTL not infused), but all six lines gave significant specific killing of the autologous LCL when compared with the HLA-mismatched target. No attempt was made to clone the CTL or to direct the antigenic specificity of the lines generated, on the basis that polyclonal lines, containing cells with specificities for a number of different EBV epitopes, would be more efficient than single clones at recognizing and eliminating EBV-carrying cells.

CTL infusion is an experimental approach to treat EBV-associated BLPD. It has evolved as a refinement to the present approach in which reduction in immunosuppressive therapy presumably promotes an increase in CTL activity and leads to tumor regression in many cases. The problem inherent with reducing immunosuppression is the risk of cell-mediated graft rejection from a concomitant increase in alloreactive CTL. This conservative approach is more acceptable in renal transplant patients where, if necessary, the graft can be sacrificed and the patient supported on hemodialysis until the tumor has regressed. However, in liver, heart, and lung recipients, this approach is more precarious, and it often proves difficult to maintain a balance between tumor destruction and graft rejection, made worse by the fear of not being able to replace the function of the failing organ. Furthermore, recurrences of BLPD commonly develop and display decreased sensitivity to conservative therapy. Cytotoxic drugs are often required at this stage, and mortality is high (7).

CTL immunotherapy has been used successfully to prevent and treat BLPD in bone marrow transplant recipients (12, 13), but the present study is the first to report potential CTL infusion therapy in solid organ transplant recipients. In this situation, the donor is usually not available to provide a starting population of T cells. Thus, autologous CTL must be grown from recipient blood samples taken before transplant, since, afterward, T cells are inactivated by continuous immunosuppressive therapy. The accumulated data from studies similar to our own now warrant a randomized controlled trial to compare CTL therapy for BLPD with the standard treatment. If CTL therapy proved the better therapy, then, for each patient undergoing transplantation, LCL and CTL would have to be stored before engraftment. However, this would require levels of funding, laboratory facilities, and workforce that are outside the reach of most transplant centers. An alternative strategy is to generate a large panel of well-characterized EBV-specific CTL from EBV-seropositive healthy individuals that would be available for use in BLPD patients on a best HLA-match basis. This strategy has the advantage of treating recipients who are EBV seronegative before transplant, and from whom CTL cannot therefore be generated, but who are at higher risk of developing BLPD if they later experience primary EBV infection.

The main risks involved in using allogeneic CTL are the induction of graft-vs-host disease and graft rejection caused by the infused CTL. However, these risks can be minimized by ensuring that the CTL are entirely EBV specific with no detectable alloreactivity in in vitro testing. In addition, in certain circumstances, a graft-vs-host effect may be beneficial to tumor regression. Recognition and destruction of the infused foreign CTL by the host immune system would probably be delayed because of the immunosuppressive drugs taken to prevent graft rejection. The proposed trial will determine not only whether CTL immunotherapy is the treatment of choice for BLPD but also whether it is worth developing as a more widespread treatment option for other opportunistic viral infections.

Acknowledgments
We thank Ms. E. Grogan and Dr. G. Miller at Division of Infectious Diseases, Yale University Medical School, New Haven, CT for checking our B95-8 cell lines for type D retrovirus, the transplant coordinators at The Royal Free Hospital for collecting blood samples, and Dr. C. Rooney, St Jude’s Children’s Hospital, Memphis, TN for her help and advice throughout the study period.

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