Determining virological, serological and immunological parameters of EBV infection in the development of PTLD

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Abstract

Post-transplant lymphoproliferative disease (PTLD) in Epstein–Barr virus (EBV) seronegative solid organ transplant recipients remains a significant problem, particularly in the first year post-transplant. Immune monitoring of a cohort of high-risk patients indicated that four EBV seronegative transplant recipients developed early-onset PTLD prior to evidence of an EBV humoral response. EBV status has been classically defined serologically, however these patients demonstrated multiple parameters of EBV infection, including the generation of EBV-specific CTL, outgrowth of spontaneous lymphoblastoid cell lines, and elevated EBV DNA levels, despite the absence of a classic EBV antibody response. As EBV serology is influenced by both immunosuppression and cytomegalovirus immunoglobulin treatment, both the EBV-specific CTL response and elevated EBV levels are more reliable indicators of EBV infection post-transplant.

Introduction

Epstein–Barr virus (EBV)-induced post-transplant lymphoproliferative disease (PTLD) that arises in solid organ transplant patients is an increasingly important clinical problem, particularly in patients who are EBV seronegative at the time of transplant. The immunosuppressive drugs required to prevent chronic organ rejection suppress the function of EBV-specific CTL, potentially resulting in the uncontrolled proliferation of EBV-infected B cells. Thus, the first line of treatment of PTLD is typically reduction of immunosuppression, as there is a causal relationship between the development of PTLD and a deficiency of EBV-specific CTL, in association with elevated EBV DNA levels (1).

Healthy individuals with a primary EBV infection rapidly become EBV IgM viral capsid antigen (VCA) seropositive after acquiring the virus [see (2) for review]. Therefore, it is assumed that upon receiving a solid organ from an EBV seropositive donor, an EBV seronegative recipient will also rapidly undergo seroconversion. Indeed, several studies have found that most EBV seronegative patients will produce EBV antibodies (IgM VCA) within 3 months post-transplant, demonstrating primary EBV infection (3–5). However, a recent single centre study in the Netherlands demonstrated that EBV seronegative lung transplant recipients developed a limited EBV antibody response, and that PTLD occurred in four of these patients prior to the development of EBV antibodies (6). A similar finding was reported by Stevens et al. (7), in which EBV IgM and IgG serology was delayed in a lung transplant patient who developed PTLD 2 months post-transplant. To address these anomalies further, the current study investigates a range of virological, serological and immunological parameters during EBV infection in solid organ transplant patients prior to and after the onset of PTLD.
Methods

Patients and samples
One hundred and fifty-five patients were enrolled in our study from 11 hospitals around Australia during 1998–2003, who either had PTLD or were at high-risk of developing PTLD after solid organ transplantation. In the Lung and Heart transplant unit at the Alfred Hospital in Melbourne, nine out of 32 high-risk patients referred to us (28%) developed PTLD. In this report, four solid organ transplant patients, from four different hospitals, presented with atypical prolonged EBV seronegativity post-transplant despite developing early-onset PTLD within 6–10 months.

Patients #2–4 were referred to our study upon the onset of PTLD, whereas patient #1 was followed longitudinally from the time of transplant. Patient #1 was treated post-transplant with tacrolimus 4 mg/bd (trough levels of 10–15 μg/l were achieved), azathioprine 50–100 mg/d, prednisolone 15 mg/d, valacyclovir 500 mg/three times daily and 1.5 million units of CMV hyperimmunoglobulin three times in the first week post-transplant, then weekly for the following 5 weeks. Gastric diffuse monomorphic large CD20+ B cell PTLD was diagnosed at 10 months post-transplant after 3 months of epigastric pain, and was treated with 50% reduction of immunosuppression, CMV hyperimmunoglobulin, oral valacyclovir 2 g four times daily, and MabThera (anti-CD20 monoclonal antibody) 700 mg weekly for 4 weeks. Patient #2 was treated with tacrolimus 0.8 mg/bd (adjunct immunosuppressive treatment had a potentiating effect, the trough levels were 5–15 μg/l) and prednisolone 10 mg/bd post-transplant. Abdominal pain and anorexia led to gastric diffuse large CD20+ B cell PTLD diagnosis 6 months post-transplant, which was resolved by decreasing immunosuppression and increasing valacyclovir. Patient #3 was treated with prednisolone 7 mg/d, valacyclovir 1 g/bd, mycophenolate mofetil (MMF) 500 mg/bd, and cyclosporine 150 mg/bd (trough levels of 800–1400 μg/l were achieved) post-transplant. Deteriorating renal function due to renal large CD20+ B cell PTLD was treated with reduction of immunosuppression, and chemotherapy. Patient #4 was treated with MMF 750 mg/bd, tacrolimus 2.5 mg/bd (trough levels of 5–15 μg/l were achieved), and prednisolone 10 mg/d, and a persistent tongue base ulcer (CD20+ PTLD) was resolved by decreasing immunosuppression. This study was approved by the respective institutional ethics committees, and clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki and in accordance with the National Health and Medical Research Council of Australia Guidelines for Research on Humans.

EBV DNA levels
DNA was extracted from isolated peripheral blood mononuclear cells (PBMCs) (3–5 × 10^6) to test for EBV DNA levels by semi-quantitative real-time PCR using primers directed to the BALF5 gene (8,9). A standard curve was generated using DNA from the Burkitt’s lymphoma cell line, Namalwa, which contains two copies of EBV per cell, and cell numbers were normalized using the albumin gene (10).

EBV serology
Plasma was collected for testing of EBV IgM VCA, IgG VCA and IgG EBV nuclear antigen (EBNA) antibodies to monitor seroconversion using common commercially available ELISA kits (PanBio, Brisbane, Australia).

ELISPOT assay
The EBV-specific CTL memory response in PBMCs was tested by interferon-γ release in an enzyme-linked immunospot (ELISPOT) assay against a range of EBV antigen synthetic peptide epitopes and the patient’s lymphoblastoid cell line derived from spontaneous outgrowth of virus-infected B cells (sponLCLs) (11,12). PBMCs (2 × 10^6) were incubated with sponLCLs at a ratio of 4:1, or with peptide (10 μg/ml) for 20 h. Spots were counted per 10^6 CD3+ T cells, as determined by flow cytometry, after subtraction of controls wells of PBMCs without peptide or with HLA-mismatch peptide, and/or sponLCLs alone.

Flow cytometric analysis
The phenotype of both PBMCs and EBV-specific CTL was determined by labelling cells with fluorochrome-labelled mouse anti-human IgG1 antibodies specific for T cell markers. Cells (2 × 10^6) were washed once in FACS buffer (1 × PBS, 2% FCS) and resuspended in 50 μl FACS buffer. Two microlitres of either CD3-FITC, CD4-PeCy5 (Beckman Coulter), CD8-PE, CD25-PE (Pharmingen, Becton Dickinson) were added to the cells and incubated on ice for 30 min, washed once, resuspended in FACS buffer and analysed on a BD FACS Calibur (Becton Dickinson).

Establishment of sponLCLs
SponLCLs were generated by plating double dilutions of 1.5 × 10^6 PBMCs in a 96-well flat-bottom plate in RPMI/10% FCS supplemented with cyclosporine (250 ng/ml). After 4–8 weeks, EBV-transformed B cells grew out spontaneously. In patient #1, sponLCLs were grown from PBMCs collected 10 weeks post-transplant upon the initial detection of EBV DNA, and again upon the onset of PTLD. SponLCLs were grown from patient #2 at 9 months post-transplant, from patient #4 at 18 months post-transplant, and were attempted from patient #3 at both 7 and 12 months post-transplant, but failed to grow.

Expansion of EBV-specific CTL
EBV-specific CTL cultures were generated as previously described (13), using the patient’s sponLCLs as stimulators. PBMCs (2 × 10^6/ml) were co-cultured with gamma-irradiated autologous sponLCLs at a responder:stimulator ratio of 25:1 for 7 days in a 24-well plate in RPMI 1640 containing 10% FCS, penicillin (100 U/ml) and streptomycin (100 μg/ml). Cultures were restimulated with autologous sponLCLs at a ratio of 10:1 on days 7 and 14, and on day 21 the culture medium was supplemented with recombinant human IL-2 (20 U/ml) (Hoffmann-LaRoche). The cultures were restimulated twice weekly, expanded, and tested for EBV-specificity between 6–8 weeks.
Defining immunological parameters in PTLD

\[^{51}\text{Cr} \text{ release assay}\]

The ability of EBV-specific CTL to induce lysis of autologous LCL was measured by specific \[^{51}\text{Cr} \text{ release}.\] Autologous LCL target cells were incubated with \[^{51}\text{Cr}\] for 1 h at 37\(^{\circ}\)C 5% \(\text{CO}_2\), washed twice, and resuspended at 10\(^5\) cells/ml in RPMI, 10% FCS. 10\(^4\) cells were added to a 96-well plate, and effector CTL were added at a 20:1 effector:target ratio. The cells were incubated at 37\(^{\circ}\)C 5% \(\text{CO}_2\) for 5 h, before pelleting the cells and 25 \(\mu\)l supernatant collected into Luma-Plate 96-well plates (Packard). The plates were dried and read on a TopCount microplate scintillation counter (Packard), and the percent specific lysis calculated by the formula:

\[
\frac{(\text{mean specific release} - \text{mean spontaneous release})}{\text{mean total release}} \times 100
\]

Results

In order to investigate the immune parameters involved in EBV immunity prior to and after the onset of PTLD in solid organ transplant patients, patients were recruited into our study who were considered to be at high-risk of developing early onset PTLD (EBV seronegative prior to transplant, and received an organ from an EBV seropositive donor). Additionally, patients were also recruited after diagnosis of PTLD, who were usually EBV seronegative prior to transplant. Four solid organ transplant patients in our study presented with atypical prolonged EBV seronegativity post-transplant despite developing early-onset, CD20-positive, EBV-positive PTLD within 6–10 months (Table 1).

Patients #2–4, who were referred to our study at the time of PTLD diagnosis, exhibited a high EBV DNA load ranging between 1600 and 29000 copies EBV/10\(^6\) PBMCs, which is typically associated with EBV-positive PTLD. Surprisingly, routine EBV serology demonstrated that these patients were EBV IgM VCA, IgG VCA and IgG EBNA negative, despite rampant EBV infection. Patient #3 became IgM VCA positive within a month of lowering immunosuppression for the treatment of PTLD, patient #4 was lost to follow up but was IgG VCA positive at 14 months post-transplant, and patient #2 was IgG VCA positive at 18 months post-transplant. ELISPOT analysis of PBMCs collected at the time of PTLD diagnosis from patients #2 and #3 indicated a dominant CTL response to EBV lytic antigens, ranging from 1500 to 2340 spot-forming cells (SFCs)/10\(^6\) CD3\(^+\) T cells. The exception to this was patient #4, in whom reactivity to specific CD8\(^+\) EBV peptides was not detected, but a strong response of 1000 SFCs/10\(^6\) CD3\(^+\) to the patient’s spontaneous outgrowth of EBV-infected B cell lymphoblastoid line (sponLCLs) was found. Thus, the serological response to EBV was delayed despite elevated EBV DNA levels, and EBV-specific CTL immunity in patients #2–4 with early-onset PTLD.

To illustrate the apparent anomaly between EBV seronegativity and the induction of other parameters consistent with EBV infection, patient #1 was followed longitudinally after receiving a lung from an EBV seropositive donor. EBV DNA was not detected in the PBMCs at 2 weeks post-transplant, but reached a peak of 9579 copies/10\(^6\) PBMCs at 11 weeks (Fig. 1A). When patient #1 was diagnosed with gastric PTLD at 10 months post-transplant, the EBV DNA level was 12785 copies/10\(^6\) PBMCs. This dropped to undetectable levels within a month of diagnosis, due to treatment with MabThera which depleted the circulating CD20\(^+\) B cells, and did not recur during the following 12 months. CD8\(^+\) EBV antigen epitopes were also tested by ELISPOT analysis to identify a specific response in available PBMC samples from patient #1, demonstrating lytic activity to GLCTLVAML (derived from BMLF1, HLA A2 restricted) in the first weeks post-transplant, which waned as the latent EBV response to HPVGEADYFEY (EBNA1, HLA B35 restricted) increased (Fig. 1B). This profile demonstrates the progression of the CTL response to primary EBV infection, concurring with other studies (9,14). Retrospectively, EBV-specific CTLs were detected by ELISPOT assay at 10 weeks post-transplant, when a strong response to the patient’s sponLCLs was initially formed and has persisted since (Fig. 1C). Interestingly, sponLCLs were also grown from PBMCs collected at this date, indicating that within 10 weeks of transplant, sufficient levels of EBV were present to transform B cells. Thus, in patient #1 the initial detection of EBV DNA coincided with the detection of EBV peptide-specific CTL in an ELISPOT assay, and sponLCLs could be easily grown from the subsequent sample.

Curiously, although patient #1 was EBV seronegative at the time of transplant, both VCA and EBNA IgG antibodies were detected within 2 weeks post-transplant, in the absence of an IgM VCA response which is typical of primary EBV infection (Fig. 1D). Over the next 2 months the VCA and EBNA IgG

### Table 1. Development of PTLD in EBV seronegative solid-organ transplant patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Transplant type</th>
<th>Onset and localization of PTLD post-tx(^a)</th>
<th>Initial detection of EBV DNA post-tx(^b)</th>
<th>Detection of EBV-specific CTL post-tx(^c)</th>
<th>EBV seroconversion post-tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>38/F</td>
<td>Lung</td>
<td>10 month, gastric</td>
<td>2 month (5089 copies)</td>
<td>2 month (356 SFC)</td>
<td>Not detected (22 month)</td>
</tr>
<tr>
<td>#2</td>
<td>35/M</td>
<td>Lung</td>
<td>6 month, gastric</td>
<td>6 month(^a) (27 918 copies)</td>
<td>6 month (2340 SFC)</td>
<td>18 month(^a)</td>
</tr>
<tr>
<td>#3</td>
<td>18/F</td>
<td>Kidney</td>
<td>6 month, kidney</td>
<td>7 month(^a) (29 000 copies)</td>
<td>7 month (1468 SFC)</td>
<td>7 month</td>
</tr>
<tr>
<td>#4</td>
<td>19/F</td>
<td>Kidney</td>
<td>8 month, tongue</td>
<td>7 month(^a) (1596 copies)</td>
<td>7 month (1012 SFC)</td>
<td>14 month(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Confirmed CD20-positive and EBV-encoded RNA positive.  
\(^b\)EBV copies/10\(^6\) PBMCs.  
\(^c\)Spot-forming cells (SFC)/10\(^6\) CD3\(^+\) T cells to EBV lytic peptides in an ELISPOT assay (11).  
\(^d\)Initial blood sample obtained from patients #2–4 at time of onset of PTLD.  
\(^e\)IgM VCA\(^+\); IgG VCA\(^+\).  
\(^f\)SFC to sponLCLs only, antigen specificity not detected.
antibody titres decreased to background levels, and the patient remained EBV seronegative at 19 months post-transplant. It was subsequently noted that the patient was treated prophylactically with purified intravenous immunoglobulin for cytomegalovirus infection (CMV Ig) during the first 6 weeks post-transplant and again after the onset of PTLD. CMV Ig is pooled from the plasma from the general population, and often contains very high titres of both anti-CMV and -EBV IgG antibodies (data not shown) (15). Thus, the detection of EBV IgG antibodies immediately post-transplant probably resulted from CMV Ig prophylaxis, rather than EBV infection. Six additional patients, who did not develop PTLD, treated with this protocol have also demonstrated transient 'false positive' EBV IgG serology in the absence of IgM VCA, which reverts to negative levels once CMV Ig treatment ceases (Fig. 2). In the representative patient shown, elevated IgG VCA and EBNA antibodies were detected in the plasma during the initial weeks post-transplant, which became undetectable at 7 weeks post-transplant after CMV Ig treatment stopped. This was not a true EBV seroconversion, as the patient became IgM and IgG VCA positive at 17 months post-transplant, demonstrating a primary EBV antibody response. The effect of the CMV Ig therapy upon EBV infection in these patients has not been examined to date.

To examine the ability to generate EBV-specific CTL from EBV seronegative transplant patients for potential immunotherapy prior to development of PTLD, PBMCs were stimulated with autologous sponLCLs, and IL-2 was added on day 21 to avoid expanding donor-specific or auto-immune CTL. EBV-specific CTLs were grown from patient #1 from three different samples collected at 11 weeks and 10 and 11 months post-transplant (Table 2). The first CTL culture comprised 86.2% CD4+CD25+ T cells which did not possess any cytolytic activity to the patient's sponLCLs. In contrast, the 10 month sample taken at the time of diagnosis of PTLD grew as a mixture of CD8+ and CD4+ T cells with limited cytotoxic capacity, whereas a month later a CTL culture of pure CD8+ CTL was generated which could recognize and kill LCL target cells (55% specific 51Cr release at a 20:1 E:T ratio). The level of CD25 expression decreased from 93% in the initial CTL cultures, to 74% in the final cultures, and may contribute to a suppressor T cell function when CD4+ cells are present. A similar phenomenon was found in patient #2, in that the initial CTL culture generated from a 9 month post-transplant sample, taken 3 months after the diagnosis of PTLD, comprised 96% CD8+ CTL, and had
improved 51Cr release against LCL target cells (52%). A similar phenomenon was also observed in EBV-specific CTL grown from patients #3 and #4 (data not shown). In these patients, CD8+ EBV-specific cultures could not be successfully grown until after the diagnosis/treatment of PTLD, indicating that the reduction of immunosuppression used to treat the disease potentially contributed to the maturation of the CD8+ response to EBV.

Discussion

This study examines in detail the progression of the immune response to EBV infection in immunocompromised solid organ transplant patients. It was found that elevated EBV levels and detection of EBV-specific CTL preceded the generation of EBV antibodies in four patients with early-onset PTLD. Additionally, the ability to generate sponLCLs from three of these patients either before or after the onset of PTLD demonstrated clear evidence of EBV infection. SponLCLs can often be grown from PTLD patients, provided that they have circulating CD20+ B cells, and elevated EBV DNA levels. In our laboratory, sponLCLs were grown from 30 out of 40 patients (75%) either at high-risk of developing, or with PTLD; however it is unknown why sponLCLs fail to grow in the remaining 25% of PTLD patients. By contrast, sponLCLs grew from only one out of 10 EBV seropositive solid organ transplant patients without PTLD, from samples collected early post-transplant when EBV DNA levels were elevated (data not shown). Thus, a sufficient immune response to EBV may not exist in vivo in EBV seronegative transplant patients to prevent the uncontrolled outgrowth of EBV-infected B cells and the subsequent development of PTLD.

The immunosuppressive agent MMF can inhibit the humoral immune response, and thus may contribute to prolonged EBV seronegativity in transplant patients (16), however only patients #3 and #4 were treated with this agent (see Methods). After commencing treatment for PTLD with the reduction of immunosuppression, patient #3 rapidly generated a primary EBV antibody IgM VCA response. Conversely, patients #1 and #2 remained EBV seronegative until 6–12 months after the development of PTLD. Thus, MMF may have delayed the EBV antibody response in patients #3 and #4. All four patients had overall IgG levels in plasma <7 g/l at the onset of PTLD (data not shown), which is considered low. Despite this, tetanus antibody levels were normal, except for patient #2, who did not have protective levels of antibody until his immunosuppression was reduced. Although IgM levels were in the normal range for all patients, and only decreased to abnormally low levels after MabThera treatment, it is likely that the immunosuppressive drugs, such as calcineurin

Table 2. Generation of EBV-specific CTL cultures from EBV-seronegative transplant patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date post-tx</th>
<th>Phenotype of culture (% of viable cells)</th>
<th>% specific 51Cr release to autologous LCLa</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CD8+</td>
<td>CD4+</td>
</tr>
<tr>
<td>#1</td>
<td>11 weeks</td>
<td>2.2</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td>10 monthsb</td>
<td>82.7</td>
<td>58.2</td>
</tr>
<tr>
<td>#2</td>
<td>11 months</td>
<td>99.1</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>6 monthsb</td>
<td>4.3</td>
<td>96.3</td>
</tr>
<tr>
<td></td>
<td>9 months</td>
<td>96.0</td>
<td>15.8</td>
</tr>
</tbody>
</table>

a:CTL:target ratio was 20:1.
b:Date of diagnosis of PTLD.

Fig. 2. Longitudinal EBV serology in an EBV seronegative transplant patient. Blood samples were collected at 0, 1, 3, 4, 6, 7, 9, 13 and 16 weeks, and 8, 17, 20 and 21 months post-transplant. The plasma was measured for IgM VCA (white bars), IgG VCA (black bars) and IgG EBNA (striped bars) antibodies, and the CMV Ig treatment doses are indicated by arrows. EBV IgG antibodies were detected during CMV Ig treatment, which disappear after 7 weeks post-transplant, and a classical EBV seroconversion was detected at 17 months post-transplant with the generation of both IgM and IgG VCA antibodies. This data are representative of six patients who were treated with CMV Ig early post-transplant.
inhibitors and steroids, contributed to low IgG levels and delayed the detection of EBV seroconversion. Conversely, EBV IgG antibody titres were increased in an EBV seronegative patient during CMV Ig treatment, in the absence of detectable IgM VCA antibodies, which may lead to a misdiagnosis of EBV seroconversion.

It has been demonstrated previously that an elevated EBV DNA load is a factor in predicting the onset of PTLD, however EBV-specific CTL responses can also contribute to this (1). In patient #1, EBV DNA was initially detected in the PBMCs at 8 weeks post-transplant, coincident with the detection of EBV-peptide specific CTL by ELISpot assay. The emerging CTL response to EBV was primarily focused to the lytic peptide, GLCTLVAML (derived from BMLF1), and decreased as the latent response to an EBNA 1 peptide HPVGEADYFEY increased. Interestingly, CTL responses to the EBNA 3, 4 and 6 latent antigens were not detected in patient #1 (data not shown), and responses to only lytic antigens and EBNA 1 are unlikely to confer a protective CTL response against EBV-infected B lymphocytes, which express high levels of EBV latent proteins. Therefore, an EBV-specific CTL response to a range of latent antigens from EBNA 3, 4 and 6 may contribute to protective immunity against the development and/or relapse of PTLD (9).

We speculate that the T cell response to EBV focuses over time, changing from a predominantly CD4+ response early in infection to a CD8+ response during latent EBV infection. This was supported by our attempts to generate EBV-specific CTL cultures from EBV seronegative patients for use as an adoptive immunotherapy to treat PTLD. Cultures grown from PBMCs taken prior to or at the onset of PTLD were CD4+CD25+ and did not possess strong cytotoxic activity to LCL target cells. However, EBV-specific CTL grown from PBMCs collected after treatment for PTLD had commenced were CD8+, cytotoxic and considered suitable for adoptive transfer to treat PTLD. This coincided with an increased PBMC response to EBV latent antigens, as tested in an ELISpot assay (data not shown). Similarly, in a previous study, EBV-specific CTL cultures grown from EBV seronegative healthy children were predominantly CD4+ and did not lyse allogeneic LCL; however, cultures grown from healthy EBV seronegative adults were CD8+ and cytotoxic (17). Interestingly, CD25+ EBV-specific CTL have been positively selected within the first 6–11 days after stimulation, and have expanded into CD4+CD25+ cultures with cytotoxic activity to LCLs (17, 18). However, these studies involved healthy EBV seropositive or seronegative donors, and may not accurately reflect the ability to generate EBV-specific CTL from immunosuppressed transplant recipients. The role of CD25 expression in CTL generated from EBV seronegative transplant patients is currently being investigated.

Previous studies have indicated that immunosuppressive treatment increases the incidence of EBV-associated malignancies, including PTLD, particularly in patients who were EBV seronegative prior to transplant. Elevated EBV DNA levels in the PBMC and plasma correlate well with EBV-positive PTLD, and are an indication of severe EBV infection. However, as demonstrated in this study, EBV serology alone cannot be used to determine the onset of EBV infection in transplant patients. Therefore, EBV infection in EBV seronegative transplant patients should be measured in multiple ways, encompassing monitoring EBV levels in PBMCs, outgrowth of sponLCLs, and testing for the presence of EBV-specific CTL. If an elevated EBV DNA load is detected in a patient in the absence of EBV-specific CTL immunity, clinicians could consider anti-viral therapy and/or reduction of immunosuppression, which are usually the first approaches used to treat PTLD. However, the risk of organ rejection is a serious side-effect to this pre-emptive treatment. Another alternative is to boost CTL immunity by administering autologous EBV-specific CTL to high-risk patients prior to the development of PTLD, as performed by Rooney et al. (19) in bone marrow transplant recipients; but this is currently not a viable treatment option in Australia. Thus, comprehensive and regular immune monitoring remains the best option for detecting PTLD quickly, as early intervention and treatment provides the highest survival outcome.

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Abbreviations

CMV Ig cytomegalovirus hyperimmunoglobulin
EBNA EBV nuclear antigen
MMF mycophenolate mofetil
PBMC peripheral blood mononuclear cell
PTLD post-transplant lymphoproliferative disease
SFC spot-forming cell
sponLCL spontaneous lymphoblastoid cell line
VCA viral capsid antigen

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