

Treatment for Epstein–Barr Virus-associated Post-transplant Lymphoproliferative Disease

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KEY WORDS

■ EPSTEIN–BARR VIRUS ■ POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE ■ TRANSPLANT RECIPIENTS ■ THERAPY

SUMMARY

The association between Epstein–Barr virus (EBV) and post-transplant lymphoproliferative disease (PTLD) has been recognized since the early days of transplantation. The major defect in the pathogenesis is the insufficient EBV-specific cytotoxic T-cell control of EBV-driven B-cell proliferations. Despite this understanding, PTLD remains a significant cause of morbidity and mortality for the transplant population. Determining the right therapy or therapies for any given patient with PTLD remains a major clinical problem. Productive areas of investigation include: identifying who will benefit from reduction of immunosuppression only; improving methods to predict those at highest risk of PTLD; developing safe and effective pre-emptive therapies; identifying who will benefit from rituximab; and developing more effective, less toxic therapies for patients with resistant or aggressive disease. Obstacles that exist are heterogeneity of disease and patient populations, and divergent approaches to immunosuppression and therapeutic interventions. Greater collaboration is needed between infectious disease specialists, pathologists, transplant physicians and oncologists to overcome such problems and develop agreed disease definitions and interventions that can be tested in large, prospective multicentre trials.

Introduction

THE ASSOCIATION OF Epstein–Barr virus (EBV) and post-transplant lymphoproliferative disease (PTLD) has been recognized since the early days of transplantation.¹ PTLD following haematopoietic stem cell transplantation (HSCT) is little different to PTLD observed following solid organ transplantation (SOT). In both conditions, PTLD may present in a very heterogeneous fashion, clinically and histologically, making disease definitions confusing and the ability to compare results of treatment difficult. In this review, pathogenesis, definitions of EBV infection/reactivation, EBV disease and PTLD, as well as known risk factors, will be discussed. However, the focus will be on the rationale and outcomes of the various therapeutic strategies for PTLD.

Pathogenesis

In a healthy individual, a very tight balance exists between EBV-infected B-cells and anti-EBV immunity, primarily EBV-specific, CD8-positive cytotoxic T-lymphocytes (EBV-CTL). To illustrate the great proliferative potential of EBV-infected B-cells, in an

immunocompetent host, only 10^{-5} to 10^{-6} B-cells are latently infected with EBV, but approximately 1–5% of all circulating CD8-positive T-cells can react against EBV.^{2–5} Following HSCT, EBV-infected B-cells in PTLD are almost always of donor origin, because host-latent EBV infection is eradicated by myeloablative conditioning regimens.⁶ Following SOT, though PTLD cells are of recipient origin, the source of EBV can be from donor, recipient or primary infection via natural oral transmission.

Results

EBV INFECTION

Detecting EBV infection in the post-transplant patient is not trivial. The ‘monospot’ test is not specific for EBV infection and antiviral serological studies have limited value, as many patients will not have the ability to respond normally and/or will have passive antibodies from gammaglobulin or blood products. The detection of EBV DNA by polymerase chain reaction (PCR) testing, though not 100% sensitive, is very specific for EBV infection.

EBV DISEASE

Following transplant, EBV infection may be asymptomatic or clinically manifest in any number of organs (Table 1).

PTLD

The diagnosis of PTLD is usually limited to lymphoid masses that are often extranodal. A particularly difficult presentation of PTLD is the very rapidly progressive, disseminated disease that clinically resembles septic

Table 1: Manifestations of Epstein–Barr virus (EBV)-related disease post-haematopoietic stem cell transplantation

- Asymptomatic infection
- Infectious mononucleosis
 - Typical
 - Fulminant, severe
- EBV hepatitis
- Lymphocytic interstitial pneumonitis
- Meningoencephalitis
- Post-transplant lymphoproliferative disease (PTLD)
 - Early lesions
 - Polymorphic PTLD
 - Monomorphic PTLD

shock or graft-versus-host disease (GVHD), which almost always results in death, with diagnosis often being made at autopsy.⁷ This very fulminant disease appears to be more common following HSCT than SOT.⁷ The World Health Organization (WHO) has classified post-transplant lymphoproliferations into three categories: early lesions; polymorphous PTLD; and monomorphic PTLD.⁸ Early lesions are not felt to be true PTLD by many, but are instead considered to be reactive hyperplasia. The presence of infiltrating T-cells, disruption of nodal architecture and necrosis are the major features distinguishing PTLD from early lesions. Histologies observed in the monomorphic subtype are similar to lymphomas observed in non-transplant patients, with the vast majority being B-cell lymphomas, although T-cell and Hodgkin's lymphomas, or even plasma-cell disease-resembling myeloma, may occur rarely. Up to 30% of PTLD following SOT will be EBV-negative and/or non-B-cell, whereas following HSCT, EBV-negative or non-B-cell disease is exceedingly rare.

A tissue biopsy is needed to make the diagnosis of PTLD. However, when mass lesions are not prominent or are inaccessible for biopsy, examination of peripheral blood, bone marrow, cerebrospinal fluid (CSF) or other body fluids for the presence of plasmacytoid cells or large B-cells that are EBV-positive may be helpful. A presumptive diagnosis of PTLD is often made in symptomatic patients with clinical or radiographic evidence of lymphoid masses in the setting of elevated EBV DNA levels in the peripheral blood; however, a diagnosis of PTLD should never be made based solely on elevated EBV viral load.

Risk Factors

In general, any factor that stimulates B-cell proliferation and/or decreases or delays T-cell immunity will increase the risk of PTLD.

DONOR/RECIPIENT AGE AND EBV STATUS

Following SOT, recipient EBV naïvety and, hence, younger age are the strongest risk factors for PTLD.^{7,9} However, in HSCT, the recipient's age and EBV status are not risk factors for PTLD, although risk increases with donor age and it has been speculated that this is due to there being fewer EBV-CTLs as age rises.⁷

TRANSPLANT GRAFTS

Following autologous HSCT, PTLD is rare⁷ and its incidence is low (about 1%) following HSCT with matched related donors (MRD), probably because of early reconstitution of EBV-CTL activity (which may be as early as 30 days post-transplant).^{7,9,10} PTLD is more common with unrelated donor HSCT.^{7,9} EBV-CTL recovery is delayed with unrelated donor use, compared with MRD use.¹⁰ However, human leukocyte antigen (HLA) disparity, which is more common in unrelated donor HSCT, may also provide chronic B-cell stimulation and proliferation, which could predispose a patient to develop PTLD. The type of solid organ allograft has also been identified as a risk factor, with low-risk patients (who include kidney, heart and liver transplant recipients) having about 1–5% risk compared with high-risk patients (namely, lung, small bowel and multiple organ transplant recipients) having a 5–15% risk of developing PTLD.¹¹

IMMUNOSUPPRESSION

In general, risk is associated with intensity of immunosuppression, particularly T-cell-specific

immunosuppression and cumulative exposure.^{11,12} Anti-T-cell antibodies are the most potent suppressants of EBV-CTL activity, followed by T-cell activation inhibitors, such as cyclosporin and tacrolimus. Corticosteroids and anti-metabolites (i.e. azathioprine, mycophenolate mofetil or methotrexate) and the rapamycin inhibitors appear to affect PTLD risk much less.¹²

Following HSCT, T-cell depletion (TCD) is the strongest risk factor for PTLD.^{7,9} TCD methods that specifically remove T-cells confer higher risk of PTLD than methods that 'pan-lymphocyte' deplete the stem-cell graft, which include CAMPATH-1, elutriation or positive CD34 cell selection.⁹ Pan-lymphocyte depletion methods decrease the number of EBV-infected B-cells and T-cells, which may delay B-cell proliferation until EBV-CTL function recovers.^{9,13}

Treatment

Successful PTLD treatment (Table 2) necessitates controlling B-cell proliferation while awaiting or ideally facilitating the development of an appropriate EBV-CTL response. Factors that contribute to the difficulty of treating PTLD patients include increased toxicity from therapy and susceptibility to infections. Additionally, non-specific enhancement of immunity increases the patient's risk of developing GVHD or allograft rejection.

Table 2: Treatment strategies for Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disease (PTLD)

Prophylactic therapies

- Antivirals – haematopoietic stem cell transplantation (HSCT), not effective; solid organ transplantation (SOT), possibly effective in high-risk groups
- Adoptive cellular therapy – HSCT only
- Donor leukocyte infusion – mixed results
- EBV-specific CTL – very effective, unavailable for most centres

Pre-emptive therapies

- EBV viral load monitoring and intervention
- Reduction of immunosuppression – SOT, perhaps effective
- Antivirals – HSCT, not effective; SOT, perhaps effective in high-risk groups
- Monoclonal antibodies (anti-CD20) – effective in HSCT; less clear for SOT

Treatment therapies

- Reduction of immunosuppression – rarely effective in HSCT; variable efficacy in SOT
- Surgical resection/radiation – effective but rarely applicable
- Adoptive cellular therapy
- EBV-specific cytotoxic T-lymphocytes – very effective in HSCT; less effective in SOT; unavailable for most centres
- Monoclonal antibodies (anti-CD20) – effective but relapses common in SOT
- Chemotherapy – effective but toxic
- Enhancement of viral replication – early results promising, needs further investigation

PROPHYLACTIC THERAPY

Since viral replication induces B-cell lysis, the use of antivirals such as ganciclovir to prevent PTLD has been questioned.¹⁴ In fact, many patients are receiving antiviral therapy when PTLD develops,^{7,15} and no studies support the use of antiviral prophylaxis to prevent PTLD following HSCT. The utility of prophylaxis following SOT is controversial. Theoretically, antivirals may play a role in reducing PTLD incidence by reducing the number of infected B-cells in high-risk patients, i.e. EBV(+) donor into an EBV(-) recipient.¹⁶

PRE-EMPTIVE THERAPY

For pre-emptive therapy to be successful, one must have a method of reliably identifying patients who are at high risk before they develop disease. Several reports demonstrate that viral load is increased at the time of PTLD diagnosis.^{17–19} There are no blinded, prospective studies to determine the predictive value of quantitative PCR for the development of PTLD. The strongest data for EBV viral load predicting patients at risk for PTLD is in the setting of recipients of TCD HSCT grafts.²⁰ Optimal timing for EBV viral load monitoring is debated, as PTLD can develop very rapidly. One must be cautious in interpreting EBV viral load since there is great variability between different methods and/or laboratories, therefore one must use the same laboratory for serial patient monitoring.¹⁹ The predictive value of EBV DNAemia can be greatly enhanced by combining it with measures of anti-EBV T-cell immunity.^{21–23} Unfortunately, these assays are not readily available at most transplant centres.

PTLD TREATMENT

Reduction/withdrawal of immunosuppression (RI) remains the gold standard for first-line PTLD therapy.¹⁴ RI is rarely successful as the sole intervention in PTLD following HSCT, because the major defect is delayed EBV-CTL recovery, not suppression of EBV-CTL function. The response to RI varies greatly (20–86%). This wide range may be attributable to different practices of RI and the wide spectrum of PTLD presentation (localized or polymorphic disease, for example) is more likely to respond to RI.^{14,24} The obvious risk of RI is increased risk of rejection or GVHD. This is most dramatically seen in cardiothoracic transplant recipients, where complete cardiovascular collapse and death has been reported in >20% of patients.²⁵

As opposed to lymphomas seen in non-transplant patients, clinical staging has not been a robust predictor of outcome, due to the propensity of PTLD to be extranodal. A consistent finding in PTLD is that ≥ 2 disease sites portends a worse prognosis.^{15,26} This seems to be due to the effectiveness of surgery in curing localized disease.²⁷ Unfortunately, resectable disease occurs in only a small percentage of patients.

Antiviral therapy has been reported to be successful in treating post-transplant EBV diseases other than PTLD (such as IM-like disease [Author Query: please define 'IM'] or meningoencephalitis). The efficacy of antiviral therapy in treating PTLD is difficult to ascertain as it is almost always used in conjunction with other potentially effective treatments (e.g. RI). A novel approach has been used recently, where viral replication is promoted with the use of arginine butyrate, which upregulates expression of EBV thymidine kinase. Ganciclovir is also given, which causes an abortive replicative cycle and no virion

production, but cell death occurs.²⁸ In this trial, of the six EBV-positive PTLD patients, five had failed chemotherapy; three of the six responded to the therapy and two achieved a complete remission.²⁸ These results are very impressive and provide a novel therapeutic strategy, requiring further investigation.

Enhancement of anti-EBV cellular immunity is an attractive approach to treat PTLD. Donor leukocyte infusions have been successful in treating of PTLD post-HSCT; however, the donor must be EBV-positive for efficacy, and severe GVHD can occur.^{17,29} To circumvent these complications, *ex vivo*-generated EBV-CTL have been shown to offer very effective prophylaxis, pre-emptive therapy and treatment for PTLD post-HSCT.¹⁸ The use of EBV-CTL in SOT is complex. Several groups have demonstrated the ability to generate autologous EBV-CTL for SOT patients with PTLD.^{30,31} As opposed to HSCT, in SOT patients these cells do not persist; repeated infusions are required. Additionally, demonstrating EBV-CTL did not correlate with reduced EBV levels.^{30,31} Since it takes 4–6 weeks to generate EBV-CTL, others have generated HLA-typed EBV-specific T-cell lines established from healthy volunteers and banked for use in treating PTLD patients, with some success.³² Although very attractive, this approach remains prohibitive for most centres due to the high level of technology, regulatory issues and cost.

Another strategy is to reduce B-cell proliferation. An anti-CD20 monoclonal antibody is commercially available (rituximab). The optimal dosing, timing or use of rituximab therapy alone (or in combination) in PTLD is being investigated. Rituximab has been used as pre-emptive therapy. One study demonstrated that, in a cohort of patients receiving TCD HSCT (using serial EBV monitoring and pre-emptive rituximab), a significant decrease in the incidence and mortality of PTLD was observed compared with a historical cohort.³³ Since EBV-CTL immunity recovers 1–6 months post-HSCT, it has become standard practice at many centres for patients receiving TCD, either *ex vivo* or *in vivo*, to be monitored by EBV PCR weekly up to 6 months post-transplant, with rituximab given pre-emptively in patients with rising or persistently positive EBV viral loads.

The use of pre-emptive rituximab in the SOT population is much more controversial. From published reports, it appears that >50% of patients with PTLD will respond to rituximab, but as many as 15–20% have a relapse/progression.³⁴ Though serious infusional toxicities are rare, one must be observant: long-term (6–12 months') B-cell depletion can occur, sometimes requiring intravenous immunoglobulin supplementation. Additionally, rituximab therapy costs between US\$5000–10 000 per infusion.³⁵

Chemotherapy has traditionally been reserved for the most refractory or aggressive PTLD cases. Benefits of chemotherapy include potent cytotoxicity against the PTLD with concurrent immunosuppression sufficient to prevent or treat allograft rejection. However, treatment-related morbidity or mortality due to end-organ toxicity and infections are problematic. For adult PTLD patients, about one-third of patients receiving chemotherapy survive, one-third die of refractory PTLD, and one-third die of therapeutic complications.^{36–38} [Author Query: we have rephrased the following sentence slightly: please check and confirm that the meaning has not changed] It is common practice to withhold chemotherapy for use only in those patients who fail rituximab.^{34,36} One study in children with PTLD following SOT used a low-dose chemotherapy approach

in which 75% of patients achieved remission and there were no deaths from therapy; however, the relapse rate was 18%.¹⁵ However, this approach has not been attempted in adult patients.

Conclusions

Associations between EBV and PTLD have been recognized since the early days of transplant medicine. Although it is clear that insufficient EBV-specific CTL control of EBV-driven B-cell proliferation is the major pathogenetic defect in PTLD development, this disorder remains a significant cause of morbidity and mortality for the transplant population. Determining the right therapy, or therapies, for any given patient with PTLD remains a major clinical problem. Productive areas of investigation include: identifying which patients will benefit from RI; improving methods for predicting high-risk patients; developing safe and effective pre-emptive therapies, identifying those for whom rituximab therapy is most likely to be of use; and finally, developing more effective, less toxic therapies for patients with resistant or aggressive disease. Obstacles that still exist are heterogeneity of disease and patient populations, and

divergent approaches to immunosuppression and therapeutic interventions. To overcome such obstacles, collaboration between infectious disease specialists, pathologists, transplant physicians and oncologists is needed. We need to develop consensus on definitions of PTLD and interventions that can be tested in large, prospective multicentre trials.

Conflicts of Interest

No conflicts of interest were declared in relation to this article.

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