Curr Transpl Rep (2016) 3:145–153
DOI 10.1007/s40472-016-0102-0

Post-transplant Lymphoproliferative Disorder (PTLD): Infection, Cancer?

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Published online: 8 April 2016

Abstract Post-transplant lymphoproliferative disorder encompasses a broad spectrum of lymphoid disorders that occur in immune-suppressed patients following solid or hematopoietic stem cell transplantation. The Epstein-Barr virus (EBV) is an oncogenic virus capable of transforming B lymphocytes and is associated with the pathogenesis of multiple benign and malignant lymphoproliferative disorders, including PTLD. This review outlines current knowledge of EBV pathogenesis, its role in B cell immortalization, transformation, and as an etiologic agent in lymphoproliferative disorders in immune-suppressed patients following transplantation. Here, we provide discussion incorporating infectious disease and medical oncology aspects.

Keywords EBV · Post-transplant lymphoproliferative disorder · EBV transformation · EBV oncogenesis

Introduction

Post-transplant lymphoproliferative disorder (PTLD) was first described in the late 1960s and remains a serious complication of transplantation today [1]. Disease incidence varies with regard to children and adults, type organ transplanted, and nature of the immune suppression regimen. The incidence in pediatric patients is higher; however, current incidence in adults ranges from 1–20 %. In adults, lower incidence is observed in kidney and liver transplant recipients, while heart, lung, and small bowel transplant recipients show much higher risk for developing PTLD [2, 3]. While major strides in the management of malignant lymphomas have been achieved over the past decade with introduction of targeted agents and monoclonal antibodies, standard preventive and therapeutic strategies for managing PTLD remain poorly characterized. The oncogenic Epstein-Barr virus (EBV) is associated with the majority of PTLD cases; however, the complex nature of this virus poses many challenges regarding its contribution to the pathogenesis of this disease.

This article is part of the Topical Collection on *Kidney Transplantation*

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Risk Factors

A significant body of investigation has been dedicated to identifying patients who are at increased risk for developing PTLD. Established risk factors include older age, type of organ transplanted, nature of immunosuppression, and EBV serostatus. Immunosuppression with cyclosporine, tacrolimus, mycophenolate mofetil (MMF), and/or anti-CD3 antibodies (OKT3 or ATG) has been shown to increase the risk of PTLD. Cyclosporine was identified in a single center retrospective study by Gao et al., to have a dose-dependent effect on incidence of PTLD [4]. Several mechanisms for this increased incidence have been introduced including reduction in key regulatory cytokines (IL-2, 3, and IFN gamma) as well as direct malignancy-promoting effects [5, 6]. Similarly, after the introduction of tacrolimus in the 1990s, the incidence of PTLD rose from 2.9 % (patients receiving cyclosporine) to 18.9 % in the case of pediatric liver transplantations in a single center analysis [7]. Furthermore, MMF has been specifically

linked to a drastic rise of observed primary central nervous system PTLD in a separate single center analysis [8].

The use of anti-CD3 monoclonal antibodies (OKT3 or ATG) for induction immunosuppression or treatment of acute rejection was reported to increase the incidence of PTLD in cardiac transplant patients from 1.3 to 11.4 %. Risk in this study was determined to be dose dependent (>75 mg); however, duration of therapy and duration of T cell suppression were hypothesized to play a large role in the development of PTLD [9]. A study reported by Opelz and Doheler, perhaps the largest retrospective analysis of PTLD in solid organ transplants (SOTs) utilizing the Collaborative Transplant Study database (approximately 200,000 transplant recipients), showed an increase from 9.3 to 39.6 % in the first year post-transplant for patients who had previously been treated with anti-CD3 monoclonal antibodies for induction or rejection therapy. Interestingly, treatment with steroids for rejection did not increase incidence of PTLD [10].

Kanakry et al. recently published a series of 762 allo-BMT patients receiving cyclophosphamide for graft versus host disease (GVHD) prophylaxis after transplant. Interestingly, zero patients developed PTLD in the first year of follow-up [11]. Similarly, the use of the anti-CD20 mAb, Rituximab, has been utilized pre-emptively to prevent PTLD when EBV viremia is detected [12]. Both studies suggest a role for regulation of B cell proliferation as a preventative strategy for PTLD.

EBV Infection and PTLD

EBV infection has been well established as a significant risk factor for the development of PTLD. In numerous studies, the incidence of PTLD has been shown to be as much as two- to threefold higher in EBV mismatch transplants (donor sero-positive/recipient sero-negative, D+/R−) and in patients who experience primary EBV infection [13–17]. In adults, the incidence of PTLD in D+/R− is known to be higher than D−/R−; however, in children, PTLD is seen in similar rate between both groups. This is likely due to the high rate of primary EBV infection in children [18, 19]. Recently, several retrospective studies have shown an increasing rate of EBV sero-negative PTLD. These studies have also shown a more bimodal distribution of PTLD, resulting in a new differentiation between early PTLD (within 2 years of transplant) and late onset PTLD. As time from transplant increases, EBV-negative PTLD becomes more prevalent and there appears to be an apparent transition in risk factors towards older age and chronic immunosuppression [20, 21].

EBV: a Transformative Virus

EBV was discovered through a collaborative effort between the British surgeon Denis Burkitt and a virology team led by Drs. Epstein, Barr, and Achong. After noting a striking correlation between the frequency of a newly described aggressive lymphoma in children and the geographic distribution of cases in equatorial Africa, Burkitt suspected a transmissible element in the disease etiology. Isolation of the virus was achieved by in vitro culture of cells obtained from lymphoma biopsies, and electron microscopy revealed the presence of viral particles [22]. Work between the Epstein and Henle labs demonstrated that EBV had the capability of transforming resting B lymphocytes in vitro [23]. Interestingly, in vitro lymphoblastoid cell lines (LCLs) could be spontaneously generated after co-culture of EBV-infected B cells with peripheral blood lymphocytes in presence of cyclosporine A. These findings suggested that T lymphocyte-mediated immune surveillance could influence the transformation and outgrowth of EBV-immortalized B cells [24].

The nature of the human adaptive T cell response has been instrumental in the understanding of both primary and established EBV infection. Primary infection leads to a brisk expansion of EBV-specific CD8+ T cells, representing up to 50 % of circulating CD8+ T cells [25–28]. Pudney et al. observed that the CD8+ T cell response following primary infection with EBV was primarily directed at the immediate early gene products, BZLF and BRLF, while showing little activation to late lytic antigen gene products suggesting that only a few select lytic gene products encoded for immune-dominant peptide targets [29]. Interestingly, CD4+ expansion does not appear to occur readily in primary infection; however, CD4+ EBV-specific T cells appear with delayed kinetics, several months after primary infection. CD4+ EBV-specific T cells seem to target latent epitopes, specifically EBNA-1, in contrast to CD8+ cells [30].

Established latent EBV infection is shaped by both CD8+ and CD4+ adaptive responses. Lytic antigen-specific CD8+ T cells represent 2–5 % of all CD8+ cells and are mostly found in the effector memory pool. CD4+ EBV-specific T cells occupy both central and effector memory pools and target latent reactivation than lytic reactivation [28, 31, 32]. EBV infection provides us with an example of how host-microbe coexistence has evolved and the importance of a finely tuned balance between host immunity and viral latency in controlling the expansion of EBV-driven B cell clones and emergence of malignant disease.

A Mechanism for Oncogenesis

EBV encodes a variety of proteins capable of orchestrating viral replication, host persistence, and transformation of B lymphocytes. Use of knock out recombinant EBV viruses has aided investigators in determining specific EBV genes (open reading frames on EBV episome) that are vital to the biology of latent and lytic (virion producing) infection. The two latent gene products that are essential for the transforming

activity of EBV are EBNA2 and LMP1 [33, 34]. Other work has established a role for additional latent gene products including EBNA1, EBNA-LP, EBNA3A, and EBNA3C in establishment and maintenance of cellular transformation. While the majority of research has focused on the oncogenic effects of latent EBV proteins, recent work by Ma et al. has identified the importance of the lytic inducing protein, BZLF1, in the transformation of B cells [35, 36]. Table 1 provides a list of critical EBV proteins and their role in oncogenesis as described below.

The first essential protein for transformation is EBNA2 protein, which functions as a transcription activator for both EBV and host cell genes. EBNA2 induces CD21 and CD23 expression on the surface of B lymphocytes and functions as a transactivator to drive LMP1 and LMP2 expression [39]. Furthermore, there is evidence supporting the ability of EBNA2 to function in NOTCH signaling and c-MYC activation [33, 40]. The second essential transformative protein is LMP1. This cell membrane protein functions in a pleiotropic manner to constitutively activate TNF receptor (TRAF) signaling resulting in increased surface adhesion protein expression. Secondarily, it up-regulates anti-apoptotic proteins BCL-2 in B cells [33, 37, 52]. Interestingly, LMP1 resembles CD40 and can partially substitute for this critical co-stimulation protein in vivo with regard to growth and differentiation of B cells [38, 53]. The driver activity of LMP1 was illustrated in a transgenic mouse model where its over expression led to constitutive activity of AKT, JNK, and NFkB and development of B cell lymphomas [54, 55].

There are several other important latent proteins in the EBV repertoire linked to inducing cellular transformation. EBNA1 is a DNA-binding nuclear phosphoprotein that functions to maintain an intracellular, latent specific episome [41, 42]. This protein acts on the latent origin of replication to induce expression of all five EBNA proteins. Overexpression of EBNA1 in transgenic mice leads to the development of lymphomas [56]. Interestingly, EBNA1 is capable of autoregulation of its own major histocompatibility complex (MHC) class 1 expression in B cells through an ubiquitin/proteosome pathway [57]. Although EBNA1 escapes MHC class I presentation, it does not escape cross presentation, with presentation to CD4+ through MHC class II [58, 59].

EBNA3 contributes towards cellular transformation through a series of transcription-regulating proteins. Of the three subtypes, EBNA3A and 3C are critical for transformation in vitro [60]. EBNA3A is a nuclear membrane protein that functions in tandem with EBNA2 to drive the NOTCH

Table 1 EBV mechanism of oncogenesis

	Mechanism of action	Role in oncogenesis	Reference
Essential latent cycle proteins			
LMP1	Plasma membrane protein which constitutively activates TNF receptors	Increased Bcl-2 levels CD40 mimicking Increased TRAF signaling	Kieff and Rickinson 2001 [33] Henderson et al. 1991 [37] Uchida et al. 1999 [38]
EBNA2	Transcription factor for both EBV and host genomes	Increased CD21/23 expression Increased LMP1/2 expression Increased Notch signaling c-MYC induction	Kieff and Rickinson 2001 [33] Kaiser et al. 1999 [34] Wang et al. 1990 [39] Sakai et al. 1998 [40]
Non-essential latent cycle proteins			
EBNA1	Binds DNA and maintains EBV episome	Increase in all 5 EBNA proteins	Gahn et al. 1989 [41] Jones et al. 1989 [42]
EBNA3A/C	Nuclear membrane protein affecting EBV and host gene expression	Increased CD21 and LMP1 expression Repress Cp promoter Possible interaction with pRb	Radkov et al. 1997 [43] Allday et al. 1994 [44] Parker et al. 1996 [45]
LMP2	Plasma membrane protein which forms TAMs	Can be utilized by the BCR proliferation signaling Can rescue a nonfunctional BCR	Fruehling et al. 1997 [46] Caldwell et al. 1998 [47]
EBERs	Small non-coding RNAs which assemble into ribonucleoproteins	Binding of protein kinase PKR which is IFN inducible and pro-apoptotic in setting of viral infection	Clemens et al. 1994 [48]
Lytic cycle proteins			
BZLF1	Transcription factor for lytic gene promoters	Increased lytic protein production Inhibits tumor-suppressor p53	Ma et al. 2012 [36]
BNLF2a	Small membrane-associated protein	Interferes with TAP-mediated peptide loading of MHC class I molecules	Ressing et al. 2008 [49]
BHRF1	Encodes a Bcl-2 like protein	Theoretical decrease in apoptosis	Young et al. 1999 [50]
BCRF1	Encodes protein with homology to IL-10 cytokine	Theoretical decrease in cytotoxic immune response due to IL-10	Suzuki et al. 1999 [51]

signaling pathway. EBNA3C is also a nuclear membrane that enhances CD21, viral LMP1 expression, Cp transcriptional suppression, and possibly interacting with pRB, a potent tumor-suppressor gene product [43–45]. The EBV genome encodes for several other nonessential pro-oncogenic proteins. LMP2A/B are cytoplasmic membrane proteins, which coalesce to form a tyrosine-based activation motif (TAM). EBV TAMs can be utilized by the B cell receptor (BCR) for signaling, a function necessary for growth and differentiation [46, 47].

While less is known about lytic proteins and their link to oncogenesis in PTLD, the lytic cycle has been shown to play an important role in transformation. Rochford et al. showed that when comparing EBV LCLs injected into SCID mice in either the lytic or latent phase, lytic positive LCLs induced tumors more rapidly [61]. Several studies have shown that the lytic cycle gp350, ZEBRA/BZLF1, can be detected in up to 80 % of PTLD tumors [62]. This suggests a role for lytic reactivation in PTLD. Furthermore, late lytic antigens are present in up to 40 % of tumors [63]. Not surprisingly, cyclosporine has been linked to lytic reactivation in vitro, resulting in EBV growth and B cell differentiation [64]. Clearly, the EBV latent protein repertoire provides multiple avenues for oncogenesis, but it appears that low-level lytic reactivation in the setting of immunosuppression may play an important role in transformation to PTLD.

PTLD: an Infectious and Malignant Disorder

PTLD encompass a broad spectrum of histologic subtypes and variety of diseases. Despite a clear association with EBV infection, PTLD remains a clinical diagnosis of transformed lymphoid cells. The World Health Organization (WHO) divided PTLD into four main morphologic categories in 2008 [65].

Classification of PTLD

Classification of PTLD is based on morphology, state of differentiation of the lymphocytic process, and clonality. Early lesions occur more frequently within the first year after transplantation and are commonly described as lesions consisting of plasmacytic hyperplasia, with immunoblasts overlying sheets of polytypic plasma cells. These changes are consistent with early forms of B cell transformation and are strongly linked to EBV infection [66]. Polymorphic PTLD, again typically an early finding, are lesions consisting of variable size lymphocytes with nuclear atypia. These lesions can be polyclonal or monoclonal in nature [65, 67]. Monomorphic PTLD remains the most common form of PTLD. This classification describes monoclonal B and T cell lymphomas. While diffuse large B cell lymphoma (DLBCL) is by far the most common histologic subtype of monomorphic PTLD, Burkitt's-like lymphoma, plasma cell myeloma, and plasmacytoma PTLD are also included in this classification [65, 68]. Classical Hodgkin's Lymphoma (cHL) is the final subtype of PTLD observed, typically presenting late after transplantation [65, 69].

Beyond WHO classifications, several studies have used stage of differentiation of lymphoid lesions to better classify PTLD. Abed et al., showed that in pediatric patients following bone marrow transplantation, PTLD tends to arise from late germinal center to early post-germinal center B cells. In this study, histologic classification did not correlate with WHO morphologic classification [70]. Furthermore, Capello et al. analyzed IgV_H rearrangements, revealing that monoclonal PTLD exists in a variety of maturation states, but most are derived from germinal center experienced B cells. This study also found a subset monoclonal PTLD IgV_H/IgV_L rearrangements which were nonfunctional. While such clones would typically be fated to undergo apoptosis, the expression of LMP1 drives survival signals promoting expansion of such germinal center lymphoproliferative processes [71].

PTLD has frequently been categorized regarding time from transplant. Early PTLD has been described as developing within the first 2 years following transplantation and late PTLD occurring after 2 years [72]. Early PTLD is associated with host EBV seronegativity at time of transplant and use of anti-T cell antibody immune-suppressive induction therapy [15, 72]. Late PTLD is associated with increased age at transplant and is more likely to present as monomorphic PTLD. Late PTLD is less likely to respond to immunosuppression reduction as discussed below and often requires management with immune-chemotherapy [72, 73].

Therapeutic Approaches to PTLD

The mainstay of initial treatment of PTLD since 1984 has been immunosuppression (IS) cessation or reduction, which is discussed below [74]. The utility of antiviral therapy is less clear and perhaps provides the most convincing evidence that PTLD represents a malignancy rather than infectious process. While acyclovir and ganciclovir demonstrate activity against lytic EBV replication, the effect on latently infected cells is limited [75]. In cases where lytic cycle induction has been achieved with agents like sodium butyrate or specific histologic subtypes of tumors (primary CNS PTLD) that show abundant expression of lytic genes (BZLF1, BXLF1, BGLF4), antiviral therapy has shown benefit [76, 77].

Reduction of Immune Suppression and Host Immune Reactivation

Reduction in IS is often the initial step in the management of PTLD. Reduction of IS alone is capable of achieving remission in up to 86 % of pediatric patients

and 40 % of adults [78, 79]. As discussed above, PTLD encompasses multiple different morphologic and histologic forms of B and T cell processes. IS reduction is superior in early and polyclonal PTLD that express highly immunogenic viral proteins, rely on oncogenic pathways driven by the latent gene products, and demonstrate low mutational burden. Monoclonal tumors that arise late after transplantation tend to show a more restricted viral gene profile and higher mutational burden allowing for other drivers of malignancy such as mutant P53 or BCL6. Despite a role for chemotherapy in aggressive disease and relapse, there is evidence to support higher response rates to chemotherapy when IS reduction is done prior to chemotherapy [80].

Response to IS reduction is observed as early as between 2 and 4 weeks when host immune cell subsets become activated and expand [81]. Early work in our lab documented the spontaneous expansion of CD8+ T cells with specific activity to BZLF1, an important lytic EBV gene following IS reduction. This expansion was associated with a prospective 91 % overall survival [82]. Furthermore, Khatri et al. showed a similar response from donor-derived CD8+ T cells in vivo against a donor-monoclonal PTLD following bone marrow transplantation. This was associated with regression of this monoclonal PTLD [83]. While long-term follow-up has yet to be reported, the low rate of relapse observed in many of these patients supports the notion that reduced IS promotes an endogenous vaccine effect and perhaps, may help guide the development of strategies to prevent PTLD in sero-negative and sero-positive patients prior to undergoing transplantation [84, 85••].

Recently, Jones et al. showed that EBNA1 (latent) specific CD8+ T cells expanded from EBV+ PTLD serum are capable of lysing EBV-transformed LCLs in vitro. In this study, there was no difference in expansion and effector function of CD8+ T cells with regard to PLTD versus healthy controls; however, there was a significant decrease in CD4+ effector T cell function for both latent EBNA1 and lytic BZLF re-stimulation in vitro [86]. This suggests preferential reduction in CD4+ effector T cell function in the setting of immunosuppression. Finally, total quantification of CD4+ T cells in PTLD have been found to be significantly lower than non-PTLD solid organ transplant recipients [87].

Chemotherapy and Rituximab

Due to lower response rates to IS reduction often seen in monomorphic PTLD, chemotherapy has been studied in relapsed or refractory PTLD. Success rates of chemotherapy such as CHOP are variable, partially due to patient selection in retrospective studies, but also due to increased toxicity from treatment. Complete response to standard chemotherapy has been reported as high at 50–75 % of cases, but this is also associated with treatment-related mortality rates of approximately 26 % [88, 89]. Given the importance of an efficient adaptive immune response, it is possible that the immune-suppressive effects of cytotoxic chemotherapy allow for more permissive escape and expansion of EBV+ clones and the high risk of relapse. Anti-CD20 antibody such as Rituximab has been used with variable success as well. In a multicenter analysis of IS reduction and front line Rituximab, OS at 3-year follow-up was 73 % compared to 33 % in patients managed with IS reduction alone [90, 91].

EBV-Specific CTL Adoptive Transfer

Perhaps the most innovative approach to PTLD management has involved the development of adoptive T cell transfer. This technology consists of the harvesting and expanding EBV-specific CD4+ and CD8+ T cells against known EBV antigens. EBV-specific T cells can be expanded indefinitely in vitro in the presence of autologous or allogeneic EBV+ LCLs functioning as antigen-presenting cells [92, 93]. Activated, primed memory T cell preparations can then be infused into patients to act as functional CTLs against EBV-associated PTLD. The rationale for this treatment is to restore the EBV-specific host immune system quickly and promote clearance of tumor. This effectively results in immediate IS reduction, without the 2- to 4-week delay for in vivo T cell expansion. Immunosuppression continues to play a role as in vivo expansion of transferred T cells will continue to be inhibited by ongoing immunosuppression to a certain degree [94]. One way to overcome time to create and expand either donor or autologous T cells, Haque et al. have generated a bank of 100 allogeneic EBV-specific CTLs, which cover most of the common HLA types [95].

In the setting following HSCT, PTLD almost invariably develops from donor B lymphocytes. Un-manipulated donor lymphocytes infused into patients have been shown to be effective in up to 70 % of patients. Unfortunately, infusion of donor lymphocytes also carries the risk of severe graft versus host disease [96]. Therefore, donor HLA-matched T cells are expanded ex vivo against LCLs for antigen-specific T cell therapy. T cell targets are against type III latency proteins, LMP1-2 and EBNA1; however, the ideal set of target antigens has yet to be determined. A recent multicenter study of EBV-specific T cell infusions in HSCT recipients showed that 11 of 13 patients with PTLD achieved sustained complete remissions with no recurrence. In this same multicenter analysis, 26 patients without PTLD were transferred T cells with a transgenic marker, which was detectable for up to 10 years in 25 of 26 patients who underwent transfer in the mid-1990s [97]. Furthermore, in a study of 10 HSCT recipients with EBV viremia, adoptive transfer prevented PTLD transformation, but did not clear EBV viremia [98].

Despite strong promise from early studies in adoptive T cell therapy, there are caveats that deserve consideration. It is important to note that autologous preparation of a patient's T cells ex vivo can be challenging due to the ongoing effect of immunosuppression [99]. Recently, Ricciardelli et al. have created EBV-specific CTLs which are resistant to calcineurin inhibitor Tacrolimus and continue to have EBV activity in the setting of ongoing immunosuppression [100••].

Conclusions

EBV infection and the development of PTLD in immunosuppressed patients provides a prime example of the multifaceted and complex nature of in vivo cellular transformation and the continual interplay between our immune system, viral infections, and cancer surveillance. Here, we have focused on the critical role of EBV infection in the development of PTLD. EBV replication and latency have a multitude of avenues to induce immortalization as well as evade the immune system. It is clear that latent EBV infection sets the stage, but chronic lytic activation in the setting of immunosuppression may contribute towards PTLD pathogenesis via infection of bystander B cells. While viral factors are capable of driving transformation, there appears to be a point at which transformed lymphocytes acquire sufficient mechanisms to drive growth and survival independently of EBV.

There has been a wealth of work completed regarding identifying which transplant patients are likely to develop PTLD. We know much about risk factors, but within these groups, we have been unable to identify which patients will go on to develop this life-threatening disease. There have been many studies examining cytokine polymorphisms as an individual risk of transformation [101–103]. In addition, there have been studies on patient HLA restriction allowing for immune evasion and transformation [104, 105•]. Jones et al. recently published data in the contrary showing no link between HLA class I associations and PTLD [106•].

It is reasonable to hypothesize that the intensity and duration of immunosuppression may significantly influence the development of PTLD. Degree of immunosuppression is difficult to quantify on an individualized basis, and controlled trials focused on systematic IS modulation based on objective immune assays that are physiologically relevant are in demand. A prospective study on CD8+ and CD4+ T cell counts and effector function with corresponding immunosuppression levels would also be of potential interest. In cases where IS reduction is not possible, prophylactic antiviral therapy or possibly prophylactic adoptive T cell transfer with calcineurin-resistant T cells are potential options to explore. Ultimately, an EBV vaccine would be of critical importance to a wide variety of patients in the pre-transplant setting [85••]. Until we can efficiently predict which patients are at the highest risk and develop novel virus-directed therapeutic or preventive approaches, PTLD will continue to lead to significant morbidity, mortality, and cost affecting a growing population of patients receiving stem cell and solid organ transplantation.

Compliance with Ethical Standards

Conflict of Interest Timothy Voorhees declares no conflict of interest. Robert Baiocchi reports a patent Methods to Treat or Prevent Viral-Associated Lymphoproliferative Disorders, issued February 2014 (#207-0536827), and a patent granted for Viral Gene Products and Methods for Vaccination to Prevent Viral Associated Diseases (granted March 2014, #2008-541493).

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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