



Biomarkers for PTLD diagnosis and therapies

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Abstract

Post-transplant lymphoproliferative disorder (PTLD) represents a spectrum of lymphoproliferative disorders and is a serious complication of pediatric transplantation. The majority of PTLD are associated with Epstein Barr virus (EBV) and the characteristic EBV+ B cell lymphomas are the leading post-transplant malignancy in children. EBV+ PTLD remains a formidable issue in pediatric transplantation and is thought to result from impaired immunity to EBV as a result of immunosuppression. However, the key viral and immune factors that determine whether EBV+ PTLD develops remain unknown. Recently, there has been much interest in developing biomarkers in order to improve and achieve more personalized approaches, in the clinical diagnosis, management, and treatment of EBV+ PTLD. Here, we review the status of immune-, viral-, and B cell lymphoma-derived candidates for biomarkers of EBV+ PTLD.

Keywords EBV · PTLD · Biomarker

Introduction

Kidney transplantation is the treatment of choice for children with a variety of end-stage renal diseases [1]. However, life-long immunosuppression is required to prevent immune-mediated allograft rejection, and this is associated with several serious complications in children, including increased risk of infection and de novo malignancies. In pediatric transplant recipients, the most common post-transplant malignancy is associated with the Epstein Barr virus (EBV). EBV is a widely disseminated gamma herpes virus that, in immunocompetent individuals, is linked with both lymphoid and epithelial malignancies including Burkitt's lymphoma, Hodgkin's disease, gastric adenocarcinomas, nasopharyngeal carcinomas, and lymphomas in immunocompromised or immunosuppressed individuals. The latter are classified within a heterogeneous collection of abnormal lymphoproliferations termed post-transplant lymphoproliferative disorder (PTLD), the majority of which are of B cell origin and are associated with EBV [2–4]. EBV+ B cell lymphomas in PTLD can be rapidly

progressing and potentially fatal, with mortality approaching 50%. In fact, EBV+ PTLD is the most common malignancy in the pediatric transplant population with the incidence ranging from ~3–7% in pediatric renal transplant recipients depending on the series [5–7]. Pediatric transplant recipients, in particular, are especially susceptible to EBV+ PTLD since they are often immunologically naïve prior to transplantation and may acquire the virus from the donor organ in the setting of maximal immunosuppression. Moreover, a notable proportion of pediatric renal recipients will develop EBV DNAemia, the clinical significance of which is enigmatic. Finally, current treatment options for EBV+ PTLD include reduction or elimination of immunosuppression, chemotherapy, radiotherapy, the anti-CD20 antibody Rituximab, and surgical resection when feasible [8]. However, there is no consensus on the optimal treatment because the efficacy of these approaches is highly variable, with relapse rates and treatment-related mortality a major concern, and because we cannot predict which treatment strategy will best benefit individual patients [9–12]. Given these challenges, there is an urgent, unmet need for the development of minimally invasive biomarkers to improve personalized diagnosis, management, and treatment of EBV+ PTLD in pediatric transplant recipients. At present, no such biomarkers have been established and validated. Here, we discuss the current state of an ongoing investigation to elucidate cellular, molecular, and genetic characteristics associated with EBV infection and EBV+ PTLD, and the possibility of their application as biomarkers (Fig. 1).

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EBV life cycle and the immune response

EBV is typically transmitted through the saliva from an infected individual to the host. In transplantation, another important mode of transmission is via the graft, particularly in children who may be EBV seronegative but receive an organ from an EBV-seropositive adult. Following infection, EBV enters a lytic phase that is accompanied by viral replication in the oropharynx involving epithelial cells and B cells that culminates in the production of new viral progeny. Viral particles are shed and can go on to infect other cells or they can be transmitted to another host. In immunocompetent individuals, the lytic phase is asymptomatic, though in adolescents and young adults it can result in infectious mononucleosis (IM), which is usually self-limiting. Eventually, EBV transitions to a chronic, latent phase in which the virus is harbored as an episome in a subset of memory B cells for the lifetime of the host. Periodically, reactivation of the virus can occur possibly triggered by antigen engagement of the B cell receptor of the infected cell; however, the exact mechanisms surrounding this process remain unclear.

There is extensive evidence that the T cell response to EBV is critical for protection against the virus [13]. Each of the phases of the viral life cycle is associated with expression of specific viral proteins that are capable of eliciting a potent cellular immune response dominated by T cells, primarily CD8⁺ T cells, although CD4⁺ T cells also play a role. Extensive analysis of T cell responses to EBV in healthy donors and IM patients, using *in vitro* assays such as ELISPOT, cytotoxicity assays, or cytokine production, and enumeration

with MHC/peptide tetramers, has catalogued a large number of HLA-restricted immunodominant epitopes derived from both latent and lytic cycle viral proteins [14]. However, the associated responses in transplant recipients, and especially those in patients that go on to develop EBV+ PTLD, are poorly understood. Nevertheless, the impairment of T cell function by immunosuppression is thought to be a major factor contributing to the development of EBV+ PTLD.

Current approaches for monitoring EBV in transplant recipients

EBV serology is routinely utilized to assess the status of viral infection, and antibodies against latent and lytic cycle proteins can be produced [15]. However, the immunosuppression administered to transplant recipients complicates the interpretation of post-transplant serologies owing to alterations or inhibition of the immune response. Consequently, PCR-based methods to directly quantitate EBV DNA are used extensively to monitor the viral load during the post-transplant period, particularly in high-risk donor-recipient combinations which occurs when EBV-naïve children receive a graft from an EBV-seropositive donor. While EBV+ PTLD can arise at any time post-transplantation, the incidence is highest in the first 1–2 years post-transplant, so monitoring tends to be most frequent during this period. In addition, the onset of clinical features symptomatic of EBV+ PTLD can prompt EBV PCR testing, and it can also be utilized to monitor response to treatment for EBV+ PTLD. Currently, there is no standardized assay for EBV quantitation and multiple PCR-based platforms have been used, including those developed in-house or at the center's designated clinical lab. Thresholds for the determination of elevated EBV viral load are established by individual labs, but this also hinders the ability to compare the results across centers. The development of a WHO international standard for EBV should contribute to improved harmonization of PCR-based quantitation of EBV across centers [16]. However, it remains unclear what the most informative compartment is to analyze for the quantitation of virus. Most studies utilize either whole blood or plasma, but others have utilized serum, peripheral blood mononuclear cells (PBMC), or isolated B cells for the assessment of EBV viral load. Thus, a multitude of studies have compared the measurement of EBV viral load in samples from various compartments [17–21] as well as in different organs [19, 21–24]. Across these studies, the viral load cutoff can vary markedly with respect to numerical range, the denominator used (per μg of DNA, per 10^5 or 10^6 cells, per ml), and the different types of organ transplants. It will also be important to evaluate this information in the context of whether the purpose is surveillance or assessment of viral load when PTLD is suspected.

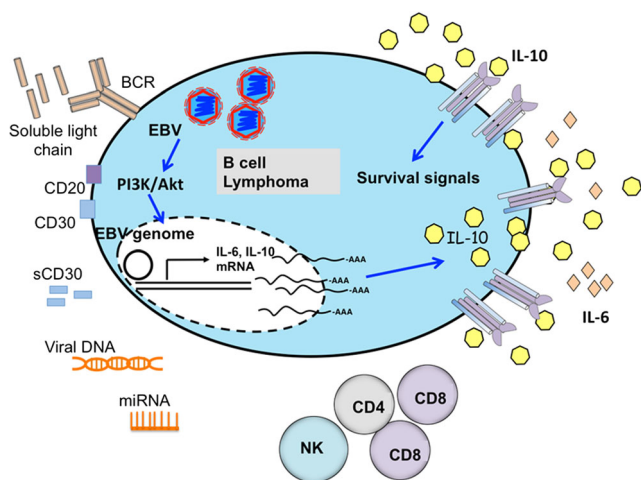


Fig. 1 Viral, immune, and lymphoma-derived biomarker candidates for EBV+ PTLD. Potential biomarkers for EBV+ PTLD include miRNA and either cell-associated or extracellular DNA from the virus; molecules, including cytokines, miRNA, soluble light chain, and sCD30 produced by the B lymphoma cells, genomic variants, or polymorphisms from the host cell or EBV DNA, and alterations in immune cell frequency or function

The clinical significance of observed elevations in EBV viral load has been the subject of intense interest in the field, yet there remains no consensus on how to apply this information to diagnosis and management of EBV+ PTLD. DNAemia does not necessarily portend the onset of EBV+ PTLD. Whereas prior studies show the majority of patients with EBV+ PTLD had elevations in EBV viral load prior to clinical diagnosis [25], and higher first positive or peak levels of virus [26, 27], not all patients with DNAemia go on to develop EBV+ PTLD. In fact, some patients manifest prolonged viral elevations and have been termed chronic high viral load (CHL) carriers, but do not progress to EBV+ PTLD [28, 29]. Green et al. showed that CHL patients were more likely to progress to EBV+ PTLD if they were cardiac recipients as compared with liver recipients, illustrating the complexity of this problem [30]. A recent study of pediatric kidney transplant recipients indicates that 8% became CHL but none went on to develop late-onset EBV+ PTLD [31]. These results suggest that the proportion of pediatric kidney recipients with CHL that ultimately develop PTLD is less than seen in other solid organ transplants. Thus, the interpretation and clinical management decisions, in the face of elevated EBV load, are challenging, and additional monitoring approaches are needed to assess the risk, or impending onset, of EBV+ PTLD.

Biomarkers

Biomarkers have been defined by the National Institutes of Health Biomarkers Definitions Group [32] as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” Biomarker assessment has become an increasingly common feature of clinical studies and may inform on the risk of disease, diagnosis, clinical outcome, and response to treatment depending on study design [33]. Less invasive sampling, as with blood, is preferable but analysis of tumor specimens in EBV+ PTLD has also been investigated. Currently, there are no established biomarkers for use in EBV+ PTLD. Discovery, validation, and reproducibility testing of biomarkers are a rigorous, multi-step process, and a variety of statistical tests must be applied to establish a biomarker as reviewed extensively by others [33, 34]. Here, we focus on recent studies of biomarker candidate molecules for determining risk, diagnosis, and response to the treatment of EBV+ PTLD. We have categorized EBV+ PTLD biomarkers candidates according to their origin, whether it be the virus itself, host genomics, the immune response to EBV, or the virally infected B lymphoma cells.

Viral nucleic acids and proteins

Detection of EBV nucleic acids and proteins in tissues for diagnosis

Viral nucleic acids or proteins are useful in establishing the presence of EBV in tissues for diagnostic purposes. Along these lines, *in situ* hybridization of Epstein Barr encoding region (EBER) is the standard method for the detection of EBV in tissue sections. The presence of EBER is consistent with the latent EBV infection, as is seen in EBV+ PTLD, and can help localize the virus to specific cell types within the lesion. Immunohistochemical staining for latent membrane protein 1 (LMP1), an EBV-encoded latent cycle protein, is also used to identify EBV-infected cells in tissue specimens, though this methodology may be less conclusive given the sensitivity of the anti-LMP1 antibodies that are an available and potentially variable expression of LMP1 in tumors.

Detection of EBV nucleic acids and proteins in blood as predictors of PTLD or at the time of diagnosis

miRNA are small, non-coding RNA that are important in post-transcriptional regulation of cellular genes and have attracted interest as biomarkers, in part, due to their stability in blood. Of note, EBV was the first virus shown to encode miRNA [35]. However, despite the growing literature on the importance of EBV miRNA in the process of B cell transformation and proliferation, there are relatively few studies that have addressed their potential as biomarkers for EBV+ PTLD. Hassan et al. [36] examined 42 EBV-encoded miRNA originating from the BART and BHRF viral open reading frames in pediatric renal transplant recipients. They noted several BART-derived miRNA, including BART7-3p, 15, 9-3p, 1-3p, and 3-3p, were only detected in CHL patients ($n = 10$ analyzed) and IM patients, compared with transplant recipients that had previously resolved an EBV infection. One CHL patient went on to develop EBV+ PTLD many years later and had markedly increased BART2-5p, compared with CHL patients, prior to disease onset. A few studies have examined the EBV miRNome within the PTLD tumor tissue itself. Navari et al. [37] found miRNA expression in tumor tissue was primarily of cellular origin, but the EBV miRBHRF-1-2 was detected in diffuse large B cell lymphoma type PTLD as compared with EBV+ Burkitt's lymphoma tissues. miRNA profiles were examined in CNS-associated EBV+ PTLD as compared with systemic EBV+ PTLD to gain insight into viral and cellular features that may distinguish them [38]. BART-encoded and BHRF1-encoded miRNA were identified in most of the EBV+ PTLD specimens but 28 host cell-derived miRNA were found to be differentially expressed between systemic and CNS type PTLD. Interestingly, the CNS PTLD specimens could be divided into two subtypes, one that was

more similar to the systemic PTLD and another that was unique to the CNS. Whereas analysis of latent cycle genes failed to distinguish the CNS and systemic PTLD specimens, at least one lytic gene could be identified in all EBV+ lymphomas, despite the presence of the type III latency pattern that is a characteristic of EBV+ PTLD.

Expression of the lytic cycle gene BZLF1 and the latent cycle gene LMP2A was analyzed in the blood of PTLD patients, transplant recipients without PTLD, and non-transplanted subjects with EBV DNAemia [39]. The ratio between LMP2A and BZLF1 was not different between PTLD and non-PTLD transplant recipients but was lower than in non-transplanted subjects. Habib et al. [40] measured the ZEBRA protein, a product of the BZLF1 gene of EBV, in the serum of 35 patients with PTLD. Soluble ZEBRA was detected in 60% of PTLD cases and was present in significantly higher levels in early-onset PTLD patients, but not late-onset PTLD, compared with subjects who did not develop PTLD.

Expression of the latent cycle genes EBNA3c, LMP1, and LMP2A/B, and the lytic cycle-associated gene gp350 was variable in the blood of 11 cardiac recipients with PTLD with a mixture of lytic and latent patterns [41]. The most common signature showed evidence of latency III and lytic cycle activity, similar to what was observed in healthy subjects with elevated viral levels but also observed in transplant recipients without PTLD. Collectively, these studies indicate that gene expression of viral genes alone does not correlate with the presence of EBV+ PTLD. Additional studies are needed to determine whether viral gene expression can be used to predict the onset of EBV+ PTLD. Along these lines, a current ongoing multi-center, prospective clinical study in pediatric transplant recipients is evaluating whether the presence of two gain of function mutations in LMP1 [42] is associated with an increased risk of EBV+ PTLD (<https://clinicaltrials.gov/ct2/show/NCT02182986>). More broadly, next-generation sequencing approaches may facilitate the identification of viral genomic variations and/or mutations that are associated with the development of EBV+ PTLD and could potentially lead to new candidate biomarkers.

Other viruses that may be informative in the diagnosis of PTLD

The Quake laboratory analyzed the human virome in cell-free circulating DNA in the plasma of 96 heart and lung transplant recipients [43], and tracked the impact of immunosuppression and antiviral prophylaxis on viral levels. Interestingly, specific viral families were associated with different levels of drug therapy. When immunosuppression and antiviral medication were low, herpesvirales and caudovirales were the predominant viral families, whereas at high drug levels, anelloviridae was dominant. Patients with biopsy-proven rejection had

significantly lower levels of anelloviruses than patients with no rejection. Together, these findings suggest that anelloviruses load may be a surrogate marker of immunocompetence with high levels of anelloviruses indicative of overimmunosuppression. Interestingly, metagenomic shotgun sequencing of formalin-fixed paraffin-embedded tissues from PTLD patients [44] showed that more than 50% of the specimens contained anellovirus sequences, and the anellovirus levels, but not EBV levels, were associated with death within 5 years in a univariate analysis. These findings indicate that changes in the virome, and even specific viruses such as anelloviruses, may provide indirect measures of immunosuppression and immune status which can promote the development of EBV+ PTLD.

Host

B cell-derived miRNA

While EBV encodes its own miRNA, it can also modulate the miRNA profile of the host cell it has infected. Harris-Arnold et al. [45] demonstrated that the establishment of a latent infection by EBV in human B cells markedly alters the expression of > 100 host cell miRNA, including miR155, which has been shown to promote the development of B cell malignancies [46]. Another miRNA, miR-194, was determined to participate in the regulation of IL-10 [45], a known autocrine growth factor for EBV+ B cell lymphomas [47]. More recently, it has been demonstrated that EBV-infected cells can release exosomes, cell-derived vesicles, containing miRNA, into the extracellular milieu suggesting that they may be a source of miRNA in the blood. Thus, like viral miRNA, EBV-infected B cell-derived miRNA are a family of molecules that warrant further exploration as biomarkers for EBV+ PTLD, particularly since miRNA have also shown promise as biomarkers in other EBV malignancies, including nasopharyngeal carcinoma [48, 49].

Genetic polymorphisms and HLA associations with EBV+ PTLD

Cytokines are important in mediating and regulating immune responses to viruses and tumor cells. Several single nucleotide polymorphisms have been defined within regulatory regions of cytokine genes that are linked to either high or low levels of production of the cytokine. Early studies examined cytokine gene polymorphisms in transplant recipients to determine whether either high- or low-production genotypes for specific cytokines were associated with the development of EBV+ PTLD. While some provocative associations were observed, some non-confirmatory or conflicting findings were also reported leaving the utility of cytokine gene polymorphism as potential biomarkers for the risk of EBV+ PTLD unresolved.

For example, IFN- γ production by T cells and natural killer (NK) cells is known to be important in antiviral responses and a genotype linked to low IFN- γ production was associated with early onset of EBV+ PTLD [50]. However, this association was not confirmed in other studies [51, 52], though associations between low-production genotypes of IL-10 and TGF- β were noted in PTLD patients [52]. Moreover, McAuley [53] reported that a specific variant allele within the promoter of TNF- α was more frequent in EBV+ PTLD patients, but no differences were seen in polymorphic regions of lymphotoxin, IL-1- α , IL-6, IL-10, TNF receptor (R) I or II, and IL-1R and IL-10R. Therefore, at present, there is no conclusive cytokine gene polymorphism or group of polymorphisms that are indicative of the increased risk of development of EBV+ PTLD.

The possible link between HLA type and susceptibility to EBV+ PTLD has also been examined. The HLA of transplant recipients could potentially impact the quality and magnitude of the immune response to EBV, due to variable efficacy in the presentation of EBV-derived peptides and recognition by antiviral effector cells. Several HLA of either donor or recipient have been linked to increased, or decreased, susceptibility to EBV+ PTLD and have been reviewed elsewhere [54–56]. These findings underscore the challenge of biomarker analyses in EBV+ PTLD owing to the complexity of the disease, the variety of organs transplanted, and the difficulty in achieving large cohort sizes that yield conclusive, broadly applicable findings.

Immune response

Identification of EBV-specific T cells and characterization of T cell responses to EBV in PTLD patients

A protective immune response to a primary EBV infection in healthy individuals is associated with the expansion of significant populations of CD4+ and CD8+ T cells that are specific for well-defined immunodominant epitopes of both lytic and latent viral genes. Eventually, the EBV-specific T cell population contracts and memory T cells are generated that provide ongoing protection to the latent, persistent virus.

Because impaired, or altered, T cell responses to EBV are thought to be an important component of the pathogenesis of EBV+ PTLD, monitoring EBV-specific T cells in transplant recipients could reveal new biomarkers for the risk of EBV+ PTLD. One possibility that has been considered is that the generation or maintenance of EBV-specific T cells is diminished in those individuals that go on to develop EBV+ PTLD. MHC/peptide multimers are complexes of HLA molecules carrying a specific peptide derived from a known immunodominant EBV epitope. These fluorescently tagged multimers, in combination with flow cytometry, can be used to identify antigen-specific T cells and, therefore, assess the

frequency of T cells for known lytic and latent antigens. Early studies with MHC class I/peptide tetramers established that pediatric liver and kidney transplant recipients without PTLD had similar frequencies of CD8+ T cells specific for peptides derived from BZLF1, EBNA3A, and BMLF1 as those reported in non-transplanted, healthy seropositive controls [57]. Moreover, seronegative transplant recipients that obtained a graft from a seropositive donor had detectable levels of EBV-specific T cells in their circulation within a month after transplantation. In adult solid organ transplant recipients, class I multimers were used to compare the frequency of CD8+ T cells specific for peptides derived from EBNA3A, BMLF1, BZLF1, and LMP2 [58]. Overall, the proportion of CD8+ T cells specific for lytic and latent cycle proteins was increased in patients with chronic viral loads in the circulation compared with patients with undetectable viral load, yet similar to the levels seen in healthy controls. Moreover, the EBV-specific T cells of patients with chronic high viral load had mixed functional characteristics including the expression of the activation marker CD38 and the exhaustion marker PD-1, along with the reduced IFN- γ production. Thus, the specificity and frequency of EBV-specific T cells are similar in PTLD-free transplant patients and healthy, seropositive donors, but questions remain concerning the functional properties of these cells.

There is limited data on EBV-specific T cells in patients with PTLD. MHC class I tetramers for EBNA3A, LMP2, BZLF1, and BMLF1 were used to assess EBV-specific CD8+ T cells in the blood of PTLD patients. The frequencies and specificities of EBV-specific T cells were similar in PTLD patients to that of healthy, EBV-seropositive recipients but lower compared with transplant recipients with EBV reactivation in the absence of PTLD [59]. The total CD4+ T cell counts were lower in PTLD patients than in a control group consisting of healthy, seropositive donors, transplant patients with EBV reactivation, or primary infection and no PTLD. Clearly, additional studies are needed to determine whether differences in the frequencies of EBV-specific T cells are informative for identifying patients at risk of PTLD. Future studies that include larger cohorts of subjects, enumeration of antigen-specific CD4+ and CD8+ T cells, and the use of multimers that expand our analysis to a broader range of HLA types and EBV epitopes are necessary.

These studies most likely will need to be complemented by the analysis of the function of EBV-specific T cells since altered or impaired T cell function could also contribute to the escape and expansion of EBV-transformed B cells. Along these lines, Jones et al. [60] investigated whether CD4 or CD8 T cells from PTLD patients could mount a functional response to EBV proteins. PBMC were obtained from PTLD patients or healthy donors and stimulated *in vitro* for 14 days with peptides from either the latent cycle protein EBNA1 or the lytic cycle protein BZLF1, then restimulated,

and the proportion of responding cells determined by flow cytometry. No differences were seen in the ability of CD8+ T cells from PTLD patients, or healthy donors, to produce IFN- γ or mobilize CD107 as a surrogate for cytotoxic function, in response to the viral antigens. In contrast, CD4+ T cells from PTLD patients had significantly reduced IFN- γ production in response to EBNA1 and BZLF1 as compared with CD4+ T cells from healthy donors. Interestingly, 10 of 14 PTLD patients carried a strain of EBV containing isoleucine in place of threonine at position 524 of EBNA. This polymorphism in EBNA1 is within a HLA-B8–presented epitope that elicits cytotoxic function by CD8+ T cells. These results suggest that variations in the EBV genome may result in alterations in the magnitude and specificity of the T cell response to the virus.

Wilsdorf et al. [61] employed a different in vitro approach to assess anti-EBV–specific T cell responses in pediatric PTLD patients by using the short-term culture of PBMC with autologous, EBV-infected B cells prior to the assessment of IFN- γ production. No differences in the proportion of T cells that produce IFN- γ were found when comparing the PTLD patients at the time of diagnosis with pediatric transplant recipients with primary EBV infection or reactivation, or with healthy controls. Nevertheless, the frequency of IFN- γ + T cells was lower in early PTLD as compared with the late PTLD (more than 12 months post-transplant). Ning et al. [62] compared the breadth of the CD4+ and CD8+ T cell response after stimulation with a panel of lytic and latent cycle peptides by assaying the cells for simultaneous production of IFN- γ , MIP-1 α , TNF- α , IL-2, and CD107 mobilization. Though only two PTLD subjects were included, they showed a striking pattern with a predominantly monofunctional response such that only one cytokine or CD107 was mobilized in each cell. In contrast, healthy subjects often showed a polyfunctional response with multiple functions produced by individual cells, a property that has been associated with immune protection against viruses. Finally, a prospective study of 45 pediatric liver recipients monitored the number of EBV-specific T cells that produced IFN- γ by ELISPOT in combination with EBV viral load [63]. All seven of the children that developed PTLD had a high viral load and a low level of EBV-specific T cells. A score was established based on the number of lymphocytes per milliliter of blood, the percentage of lymphocytes in PBMC, and the number of spots per 10^5 PBMC in the ELISPOT. No patients with a score of greater than $2/\text{mm}^3$ developed PTLD. Together, these studies suggest that the combined assessment of viral load with T cell frequency and function may hold potential in identifying patients at risk of developing EBV+ PTLD.

Natural killer cells and control of EBV infection

NK cells are a rapidly responding cellular effector population of the innate immune system that is particularly important in the elimination of virally infected cells and tumor cells. Recent studies [64, 65] have revealed that NK cells play an important role in the recognition and cytotoxicity of B cells that harbor a latent EBV infection, as is seen in the EBV+ B cell lymphomas associated with PTLD. The phenotypic analysis demonstrated that NK cells in PTLD patients had predominantly CD56^{dim}CD16[−] and CD56[−]CD16⁺ NK cells in contrast to asymptomatic pediatric transplant [66]. Furthermore, NK cells in PTLD patients had downregulation of the natural cytotoxicity receptor NKp46 and the activating receptor NKG2D and upregulation of PD-1, associated with cellular exhaustion, suggesting NK cells from PTLD patients are functionally impaired. Taken together, these studies indicate that further investigation of NK cell subsets may be informative in identifying PTLD-associated changes in the immune response to EBV.

B lymphoma cells

EBV+ B cell lymphomas–derived cytokines associated with PTLD

Establishment of latent EBV infection in B cells results in multiple changes in host cell gene expression that promote tumor cell survival and proliferation. One latent cycle protein, LMP1, can induce expression of host cell cytokines including IL-6 [67] and IL-10 [68]. These cellular cytokines are secreted by the infected B cells, utilized as autocrine growth factors [47, 67], and can be measured in the blood of PTLD patients. Thus, they have received interest as possible biomarkers of EBV+ B cell lymphomas in PTLD. Most studies have been done in adult transplant recipients with PTLD and have shown increases in IL-10 levels at the time of EBV+ PTLD diagnosis [69–71], whereas some also show increases in IL-6 at the time of diagnosis [70] and others do not [71]. IL-6 has also been shown to increase prior to the development of PTLD in adults [67] and children [72], and seems to track more closely with disease showing a rise when the disease progresses and a drop in levels with successful treatment [70]. However, elevations in IL-6 are also noted in patients with other infections or rejection [67].

Other soluble molecules produced by B cell lymphomas

CD30 is a member of the TNF receptor family and is expressed on the membrane of activated T and B cells. Soluble CD30 is released when CD30 is proteolytically cleaved from the membrane and has been detected in the circulation following viral infection, graft rejection, cancer, and

autoimmunity. In a single study of 23 EBV+ PTLD patients, sCD30 was elevated during PTLD compared with transplant recipients without PTLD and healthy controls [73]. The elevations in sCD30 in PTLD patients correlated with increased EBV DNA levels. Another product of B cells, free light chain (FLC) of immunoglobulin (Ig), is also released by B cells and elevated light chains or altered kappa/lambda ratios in the circulation may be an indication of abnormal B cell activation or expansion. Elevations in FLC were noted in adult EBV+ PTLD patients but a significant proportion of matched PTLD-free transplant controls also showed elevations in FLC. Nevertheless, elevated FLC in the peripheral blood prior to EBV+ PTLD were associated with an increased risk of subsequent disease [74]. The same group conducted a study of 36 liver and hematopoietic stem cell pediatric transplant recipients, including 12 with PTLD, and analyzed for kappa and lambda FLC, monoclonal Ig (M) proteins, and sCD30 [75]. More than 90% of the samples had elevations in sCD30 and 20–30% showed elevations in kappa or lambda FLC. M proteins were found in the majority of subjects but were more frequent in the PTLD samples. Overall, these studies indicate that in transplant recipients, disturbances in B cells including elevated FLC are common. Similarly, sCD30 can be released in response to a variety of antigenic stimuli. Thus, other immune-activating events beyond EBV infection and the pathogenesis of PTLD modulate the levels of these B cell-associated markers.

Summary

Diagnosis, management, and treatment of EBV+ PTLD remain a clinical challenge. At present, there is a need for non-invasive, specific, and sensitive biomarkers that can contribute to improved clinical care. Research to identify new biomarkers has primarily focused on molecules of viral, immune, or lymphoma origin that can be detected in the blood. While there are some promising candidate molecules, there are significant challenges with identifying biomarkers that are specific indicators of risk for development of EBV+ PTLD, diagnosis, and response to treatment. The improved utility may be achieved by a combined analysis of candidate molecules with measurements of viral load. In addition, the majority of studies to date have been conducted in comparatively small numbers of adult subjects. Therefore, it will be important to establish multi-center pediatric studies to increase the study cohort size in order to evaluate and validate the most informative biomarkers.

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

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