# Epstein-Barr Virus-Associated Post-Transplant Lymphoproliferative Disorder

Michal Bar-Natan MD and Arnon Nagler MD MSc

Hematology Division, Sheba Medical Center, Tel Hashomer, Israel

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Epstein-Barr virus-associated post-transplant lymphoproliferative disorder is a serious life-threatening complication after hematopoietic stem cell transplantation and solid organ transplantation. It represents a heterogenous group of abnormal lymphoid proliferation, generally of B cells, that occur in the setting of ineffective T cell function because of immunosuppressive treatment and/or state [1].

The spectrum includes benign conditions such as infectious mononucleosis-like illness, polyclonal lymphoid hyperplasia, to monoclonal malignancies such as aggressive B cell lymphomas [2]. The Epstein-Barr virus is a latent herpes virus that infects > 90% of the world's population. After primary infection in immunocompetent hosts the EBV genome forms an episome that remains latent throughout life in resting memory B cells [3,4]. This latency is tightly regulated by the cellular immune system, consisting of specific cytotoxic T lymphocytes (CD8+) as well as specific CD4+ lymphocytes [1,3].

## Pathogenesis

EBV was the first human virus implicated in oncogenesis. All EBV-positive malignancies including Burkitt`s lymphoma, gastric adenocarcinoma, nasopharyngeal carcinoma, Hodgkin's disease and peripheral T/natural killer cell lymphoma are associated with the virus latent cycle [4,5].

Four patterns of EBV latent gene expression have been described: types 0, I-III [5,6]. In type 0 latency only the viral protein-latent membrane protein 2 (LMP2) is expressed. In type I latency only EBV nuclear antigen (EBNA)-1 and BARF0 are expressed; type II latency is characterized by EBNA-1, BARF0, LPM1 and LMP2 expression. EBV-PTLD is associated in most cases with type III latency, which is characterized by expression of the entire array of nine EBV latent proteins (EBNA 1, 2, 3A, 3B, 3C, (leader protein) LP, BARF0, LMP1 and LMP2). In all types of latency the EBV-derived polyadenylated viral RNAs, being EBERs 1 and 2, are expressed. EBNA2 up-regulates the expression of EBV LMP1 and LMP2, as well as cellular proteins that contribute to the growth and transformation of B cells [3]. LMP1 is a major transforming protein of EBV, behaving as an oncogene. It has pleiotropic effects when expressed in cells, resulting in the induction of cell surface adhesion molecules and activation antigens, up-regulation of anti-apoptotic proteins, and stimulation of cytokine production [4]. LMP1 induces signaling response in cells that mimic a constitutively active form of the B cell surface molecule CD40. It binds to several of the tumor necrosis factor receptor-associated factors both *in vitro* and in EBV-positive lymphoma [3]. The non-translated types of EBV-encoded RNA (EBER) do not encode proteins, but they may be important for oncogenesis and resistance to programmed cell death or apoptosis [3].

T cells are the counterparts for the development of PTLD. When T cell function is compromised, the control of infected B cells is impaired, leading to an increase in their number and unchecked proliferation [2,6]. The importance of T cell dysfunction in the pathogenesis of PTLD is highlighted by the fact that the majority of PTLD cases occur within the first 6 months after transplantation when T cell deficiency is most profound [7].

## Incidence and risk factors

The incidence of PTLD after allogenic HSCT is approximately 1%. The incidence is significantly increased by the following risk factors: the use of an HLA mismatched family member or matched unrelated donor; T cell-depleted donor cells; intensive immunosuppression with T cell antibodies for prophylaxis/treatment of graft versus host disease such as antithymocytic globulin or anti-CD3; and an underlying diagnosis of primary immunodeficiency. The incidence may reach up to 22%, with more than three major risk factors [7]. The risk of developing PTLD after solid organ transplantation is highest in the first year after transplantation. The incidence varies with the type of organ transplant. Risk factors include prolonged and extensive immunosuppression and EBV-naïve state at the time of transplant [1].

## Clinical presentation

The clinical presentation is often non-specific and includes a diverse spectrum of signs and symptoms, ranging from fever, sweats, malaise, lethargy, cervical lymphadenopathy and enlarged tonsils to involvement of any organ including lung (nodules,

EBV = Epstein-Barr virus

PTLD = post-transplant lymphoproliferative disorder
HSCT = hematopoietic stem cell transplantation

consolidations, dysfunction), liver (hepatitis), spleen, kidney, bone marrow, intestine (vomiting, diarrhea), and central nervous system (seizures, headaches and focal neurologic lesions). Sometimes it presents as fulminant sepsis or severe GVHD [1,8].

## Diagnosis

Because the clinical presentation is non-specific and the differential diagnosis wide, a high index of suspicion is needed, and the diagnosis requires tissue biopsy confirmation with the demonstration of EBV DNA, RNA or proteins in biopsy tissue [2,3].

Laboratory findings often include abnormal blood count (leukopenia, atypical lymphocytosis, anemia), elevated lactate dehydrogenase and uric acid. Immunoglobulin levels may be elevated. Imaging tests are helpful in evaluating the patients; computerized tomography scan can show lymphadenopathy, pulmonary nodules, and other extranodal site involvement (spleen, kidney, liver) [8]. There is tremendous interest in developing tests that will predict the development of PTLD. It was shown that PTLD after solid organ transplantation or HSCT is associated with a rise in EBV-DNA load detected by polymerase chain reaction-based methods in peripheral blood samples.

Recently it was shown that EBV reactivation is a common occurrence after transplantation (up to 68% in one series) [9] and only a proportion of patients (7% in this series) will progress to PTLD. Patients with high levels of reactivation and more episodes of reactivation were more likely to progress to PTLD.

Monitoring EBV-specific cytotoxic T lymphocyte response is feasible (but not routinely performed) and may be useful in assessing the risk of PTLD development in patients with increased EBV-DNA viral load [6].

## Treatment and prevention

There is no consensus regarding the optimal treatment for PTLD. A dose reduction or termination of immunosuppressive therapy is the first step in solid organ transplant patients. It can lead to partial or complete regression but carries the risk of graft rejection [1,8].

Antiviral drugs, such as acyclovir, that inhibit the active replication of other herpes viruses have limited therapeutic value for established PTLD because PTLD is associated with the latent cycle of EBV infection, in which B cell proliferation is independent of spontaneous viral replication [2,8].

Infusion of donor lymphocytes resulted in a high response rate but carries a significant risk of GVHD, which can be fatal [6,10]. In order to reduce this risk, the use of *in vitro* expanded antigen-specific cytotoxic T lymphocytes has been tried. Recently, 60 high risk HSCT patients were infused with donor-derived EBV-specific CTL. The infusions were well tolerated and none of the patients developed PTLD. Moreover, CTL infusion resulted in a 2–3 log decline of EBV-DNA load. Furthermore, five of six patients who received CTL as treatment for overt PTLD achieved complete remission [1,6].

Another promising therapeutic option to control B cell proliferation is anti-B cell antibody therapy. Rituximab, a chimeric murine/human monoclonal anti-CD20 antibody, has been used as prophylaxis and treatment for PTLD in many retrospective studies, with a response rate that varied between 60 and 100% [1,11]. In a recently published prospective study of 43 PTLD patients after solid organ transplantation, the response rate to rituximab monotherapy reached 44.2% at day 80. The response was maintained in 68% of the patients after 1 year [11]. Side effects include profound and prolonged B cell depletion for 6–8 months, which may exacerbate the immunodeficiency in transplant recipients and expose the patients to infections. It is also possible that monoclonal antibody therapy may result in the selection of B cells negative for the target antigen and outgrowth of CD20-negative PTLD, as reported in some lymphoma patients after anti-CD20 therapy [12].

In this issue of *IMAJ*, Shilon et al. [13] describe two children after liver transplantation with PTLD presenting in the upper respiratory tract. The incidence of PTLD in children has been reported as high as 8%, and varies according to the organ transplanted: lung/heart-lung 10–20%, liver 5–14%, heart 4–10%, kidney 1–10% (nearer 1% in most series) [8]. It is known that young age, primary EBV infection after transplantation, and EBV-positive donor organ placed into a EBV-naïve recipient are the three major risk factors for PTLD development in children [14]. Both children in this case report were young (18 and 14 months) and had evidence of primary infection after transplantation (elevated immunoglobulin M antibodies dropping with therapy).

Although any organ system may be involved at presentation, reports from the literature have found the abdomen to be involved in 60–70%, the thorax in 45–65%, the head and neck in 20–40% and brain in 1–10% [15,16]. Disease manifestations in the head and neck area usually include diffuse enlargement of the tonsils and adenoids, cervical adenopathy and paranasal sinus involvement, but can appear in any location of the head and neck [16]. Physicians should therefore maintain a high index of suspicion as these manifestations sometimes mimic common diseases, and early diagnosis and treatment leads to better outcome.

## Conclusions

PTLD is a rare but devastating disease. Over the last decade, effective immunotherapies for PTLD have been developed, including donor-derived EBV-specific CTL and monoclonal antibodies like rituximab. Moreover, routine surveillance of patients by measuring the EBV-DNA load in peripheral blood samples has proved helpful in identifying patients at high risk for developing PTLD, particularly in the presence of other known risk factors. In the future, laboratory tests assessing EBV-specific T cell function may become available to assist in the management of patients at risk. Patients with imminent PTLD could then receive a preemptive therapy.

GVHD = graft versus host disease
CTL = cytotoxic T lymphocytes

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**Correspondence:** Dr. M. Bar-Natan, Hematology Division, Sheba Medical Center, Tel Hashomer 52621, Israel.
Phone: (972-3) 530-5830
Fax: (972-3) 530-5377
email: michal.barnatan@sheba.health.gov.il