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REVIEW

Epstein Barr virus-associated lymphoproliferative diseases: the virus as a therapeutic target

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Epstein Barr virus (EBV)-associated lymphoproliferative diseases (LPDs) express all EBV latent antigens (type III latency) in immunodeficient patients and limited antigens (type I and II latencies) in immunocompetent patients. Post-transplantation lymphoproliferative disease (PTLD) is the prototype exhibiting type III EBV latency. Although EBV antigens are highly immunogenic, PTLD cell proliferation remains unchecked because of the underlying immunosuppression. The restoration of anti-EBV immunity by EBV-specific T cells of either autologous or allogeneic origin has been shown to be safe and effective in PTLDs. Cellular therapy can be improved by establishing a bank of human leukocyte antigen-characterized allogeneic EBV-specific T cells. In EBV+ LPDs exhibiting type I and II latencies, the use of EBV-specific T cells is more limited, although the safety and efficacy of this therapy have also been demonstrated. The therapeutic role of EBV-specific T cells in EBV+ LPDs needs to be critically reappraised with the advent of monoclonal antibodies and other targeted therapy. Another strategy involves the use of epigenetic approaches to induce EBV to undergo lytic proliferation when expression of the viral thymidine kinase renders host tumor cells susceptible to the cytotoxic effects of ganciclovir. Finally, the prophylactic use of antiviral drugs to prevent EBV reactivation may decrease the occurrence of EBV+ LPDs.
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INTRODUCTION

Epstein Barr virus (EBV) is the first human virus isolated from a neoplastic disorder.[1] EBV infects most humans. The initial infection may be asymptomatic and often occurs in childhood. In older individuals, a more florid clinical syndrome of infectious mononucleosis may occur.[2] Regardless of the initial manifestation, EBV establishes a lifelong latency in B cells. EBV exists in an episomal form and is not integrated into the host cell genome.[2] EBV is involved in numerous malignancies (Table 1), suggesting that it plays an important role in oncogenesis.

During primary infection, EBV enters the oropharyngeal epithelial cells. Viral replication leads to infection of naïve B cells. EBV-infected B cells become lymphoblasts and express the entire EBV latency gene complex, which consists of at least 10 proteins (which include EBNA1, EBNA2, EBNA3, LMP1, LMP2 and BARF1) and two small RNAs (type III latency).[3] These lymphoblasts are highly immunogenic and are targets of EBV-specific cytotoxic T cells. However, when EBV-infected B cells enter lymphoid follicles, downregulation of these EBV immunogenic proteins occurs, with the expression of three less immunogenic EBV proteins (EBNA1, LMP1 and LMP2) (type II latency) remaining, thereby allowing these EBV-infected B cells to survive. When memory B cells exit lymphoid follicles, they may not express EBV-related proteins (type 0 latency). These memory B cells circulate and re-enter secondary follicles, where they express EBNA1 (type I latency). EBNA1 promotes the replication of the viral episome.[4] The persistence of EBV-infected B cells of type II latency in these secondary lymphoid tissues, including the tonsils,[5] promotes the expression of LMP1 and LMP2, which are essential signals for the survival of these circulating EBV-infected memory B cells.

A dominant theory in oncogenesis is that when transformation occurs, neoplastic cells are often arrested at their respective stages of cellular development or maturation. Transcriptional programs are often retained, and phenotypes of these neoplastic cells often resemble those of their normal counterparts. This notion is reflected in the various EBV latency states in EBV-associated malignancies (Table 1).

EBV-ASSOCIATED LYMPHOID MALIGNANCIES

The number of EBV-associated lymphoid malignancies continues to increase (Table 1), emphasizing the importance of

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Table 1 Epstein Barr virus-associated malignancies

Neoplasms	*EBV latency*	*Treatment strategy*	*EBV-targeted therapy*
Lymphoproliferative diseases			
B cells			
Post-transplantation lymphoproliferative disorders	III	Reduction of immunosuppressive agents; rituximab +/− chemotherapy	+++
Lymphoproliferative diseases associated with primary immune disorders	III	Rituximab +/− chemotherapy	−
Lymphomas associated with HIV infections	III	HAART and chemotherapy	−
Iatrogenic immunodeficiency-associated lymphoproliferative diseases	III	Reduction of immunosuppressive agents; rituximab +/− chemotherapy	−
EBV+ diffuse large B-cell lymphoma of the elderly	II	Rituximab+CHOP-based chemotherapy	−
Diffuse large B-cell lymphomas, not otherwise specified	II	Rituximab+CHOP-based chemotherapy	++
Pyothorax-associated lymphoma	III	Rituximab+chemotherapy	−
Plasmablastic lymphoma	III	Rituximab+chemotherapy	−
Primary effusion lymphoma	I	Rituximab+chemotherapy	−
Burkitt lymphoma	I	Intensive chemotherapy	+
Hodgkin lymphoma	II	Combination chemotherapy	++
T cells			
Peripheral T-cell lymphoma, not otherwise specified	II	Chemotherapy	++
EBV+ lymphoproliferative disorders of childhood	I/II	Chemotherapy	−
Chronic active EBV infection (T-cell type)	II	Allogeneic HSCT	−
NK cells			
Extranodal NK/T-cell lymphomas, nasal type	II	L-asparaginase-based chemotherapy	++
Aggressive NK-cell leukemia	II	Chemotherapy	−
Chronic active EBV infection (NK-cell type)	II	Allogeneic HSCT	−
Epithelial cancers			
Nasopharyngeal cancer	II	Radiotherapy +/− chemotherapy	++
Lymphoepithelioma-like carcinoma	II	Surgical resection +/− chemotherapy	−
Gastric carcinoma	I	Surgical resection +/− chemotherapy	−
Sarcomas and other soft-tissue tumors			
Inflammatory pseudotumor variant of follicular dendritic cell sarcoma	II	Chemotherapy; surgical resection	−
HIV-related smooth muscle tumor	III	HAART; surgical resection	−
Post-transplantation smooth muscle tumor	III	Reduction of immunosuppressive agents; surgical resection	−
Congenital immunodeficiency-related smooth muscle tumor	III	Surgical resection	−

Abbreviations: EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; NK cells, natural killer cells.
+++: most experience; ++: some experience; +: limited experience; −: no experience.

EBV in lymphomagenesis. EBV-associated lymphoproliferative diseases (LPDs) can occur both in immunocompetent and immunocompromised patients. In general, EBV latency states correlate with immunocompetency. EBV type III latency is typically found in LPDs developing in immunodeficient subjects, whereas type I and II latencies are observed in LPDs developing in otherwise immunocompetent subjects.

EBV+ LPDs are highly heterogeneous with regard to pathology and the host background against which the diseases arise. Therefore, treatment strategies are also highly variable.[6] In general, chemotherapy is the mainstay of treatment in immunocompetent patients. In B-cell malignancies, the anti-CD20 monoclonal antibody rituximab is also administered. In immunodeficient patients, efforts to boost the host immunity should be attempted first. In patients with iatrogenic immunosuppression, including autoimmune diseases or after organ allografting, decrease or withdrawal of the immunosuppression constitutes the first-line strategy. If the LPDs fail to regress, the

use of chemotherapy is indicated. In patients infected with human immunodeficiency virus, highly active antiviral treatment should also be initiated together with chemotherapy.[7]

In patients not responding to these traditional approaches, strategies targeting EBV have been adopted.

CELLULAR THERAPY TARGETING EBV-POSITIVE LPD WITH EBV IN TYPE III LATENCY

EBV+ neoplastic cells express EBV antigens, which are potential targets for EBV-specific cytotoxic T cells. However, cellular control of EBV-infected cells might not be effective when the immune system is suppressed. The prototype of this condition is post-transplantation lymphoproliferative diseases (PTLDs). This condition can occur after two types of organ transplantation: allogeneic hematopoietic stem cell transplantation (HSCT) and solid organ allografting. After allogeneic HSCT, lymphoid cells are donor-derived. Therefore, PTLDs are of donor lymphoid origin. After solid organ allografting, however, lymphoid cells are derived from the recipient. Hence, PTLDs are of recipient origin.

EBV-POSITIVE PTLD AFTER ALLOGENEIC HSCT

PTLD is an important complication after allogeneic HSCT.[8] Risk factors include T-cell depletion, anti-thymocyte globulin use, unrelated or human leukocyte antigen (HLA)-mismatched grafts where T-cell depletion or anti-thymocyte globulin is used, age $\geqslant 50$ years and second HSCT.[9] The incidence of PTLD peaks at 2–3 months, primarily within the first 6 months post HSCT.[9] Once developed, PTLD post HSCT is a highly fatal disorder.

Early detection of EBV+ PTLD offers the best possibility of cure. In at-risk patients, monitoring circulating EBV DNA loads enables the early detection of impending EBV+ PTLD.[10–12] Once an increasing trend of circulating EBV DNA is detected, reduction or withdrawal of immunosuppression should be instituted.[8] Because most cases of EBV+ PTLD post HSCT involve CD20+ B cells, the preemptive use of rituximab has been shown to abort the development of clinical disease.[8,12,13] However, when PTLD has developed, the use of rituximab and chemotherapy is less effective. In addition, mortality remains high, suggesting that alternative approaches should be pursued.

The earliest evidence that cellular therapy might be effective for EBV+ PTLD post HSCT was provided by effective disease control with donor lymphocyte infusion, which re-constituted the recipient with donor-derived EBV-reactive T cells.[14] However, donor lymphocyte infusion exacerbates graft-versus-host disease and is not always practical in severely ill patients. Donor lymphocytes may not be available from matched-unrelated donors and almost certainly cannot be obtained from cord blood donors.

The production of EBV-specific cytotoxic T cells provides a more specific and potent means of targeting EBV+ PTLD.[15] Observations in experimental animals showed that T cells primed against EBV homed to and induced selective regression of EBV-infected B cells.[16] Furthermore, genetically marked donor-derived EBV-specific T cells persist for long durations and re-constitute immunity against EBV in recipients of allogeneic HSCT.[17] These observations ushered in initial trials where HSCT recipients either at risk of or who had actually developed EBV+ PTLD were adoptively transferred with EBV-specific T cells.[10,18,19] These studies showed that infusions of donor-derived EBV-specific T cells were safe and did not induce graft-versus-host disease.[10,18,19] These preliminary observations were confirmed by two recent studies. In 101 patients who received donor-derived EBV-specific T cells prophylactically, none developed PTLD. In 13 patients who were treated for established EBV+ PTLD, 11 responded to EBV-specific T cells.[20] In 47 patients with established EBV+ PTLD receiving either donor-derived or third-party EBV-specific T cells, an overall response rate of 68% was achieved.[21] Non-respondents harbored EBV strains that differed from those against which the T cells were primed. In one exceptional case, the PTLD was recipient-derived, so that donor-derived T cells were ineffective owing to HLA restriction.[21]

The key findings of these studies are that the prophylactic use of donor-derived EBV-specific T cells is safe and effective in allogeneic HSCT patients at high-risk of EBV+ PTLD. However, the use of cellular therapy has not been compared with immunotherapy using rituximab, so that its role in prophylactic settings remains undefined. In patients with established EBV+ PTLD, wherein treatment with rituximab and chemotherapy exhibits a low successful outcome, EBV-specific T cells can also achieve a high response rate.

EBV-POSITIVE PTLD AFTER SOLID ORGAN ALLOGRAFTING

PTLD after solid organ allografting occurs considerably later, typically after the first year post-transplantation, although cases may occur after many years.[22] The majority of PTLD cases after solid organ allografting are derived from B-cell lineage, which may or may not be EBV+. A minority of cases is derived from T-cell lineage and is typically EBV-negative.

For EBV+ PTLD after solid organ allografting, a strategy of decreased immunosuppression followed by the use of rituximab and chemotherapy currently serves as the standard approach.[23] However, not all patients respond, and alternative approaches are needed for refractory patients.

Early observations indicated that cellular therapy might be useful for EBV+ PTLD after solid organ allografting.[24,25] These studies utilized fully or partially HLA-matched allogeneic T cells. Later studies examined the use of autologous T cells. In patients with evidence of active EBV infection, prophylactic infusion of autologous EBV-specific T cells was safe and appeared to prevent PTLD.[26,27] The use of autologous EBV-specific T cells in conjunction with withdrawal of immunosuppression and immunochemotherapy had also been shown to be feasible and effective.[27,28]

Although these studies have indicated that autologous EBV-specific T cells are safe, do not cause graft rejection and may be effective especially when used prophylactically, the

advent of rituximab and better chemotherapy regimens means that this strategy is only reserved for patients not responding to standard approaches.

THE USE OF THIRD-PARTY EBV-SPECIFIC T CELLS FOR PTLD

The generation of EBV-specific T cells, either allogeneic or autologous, requires 8–12 weeks.[3] Therefore, the timely treatment of a patient with active disease not responding to conventional therapy is problematic. Furthermore, in cord blood or matched-unrelated donor HSCT, donor-derived T cells are generally not available. In patients after solid organ allografting treated with immunosuppression, the collection of enough autologous T cells may not always be feasible.

The use of third-party partially HLA-matched EBV-specific T cells has been explored.[25] In an early study, eight patients received EBV-specific T cells selected on a best HLA-match basis, with three patients achieving a complete remission.[29] A multicenter study examined 33 patients treated with the same strategy. The overall response rate was 52% at 6 months.[30] Similarly, third-party EBV-specific T cells have also been used successfully in PTLD after cord blood transplantation,[31] wherein donor-derived T cells could not be obtainable.

The logistics of third-party EBV-specific T cells has recently been simplified by the establishment of a bank of HLA-typed allogeneic EBV-specific T cells using good manufacturing practice standards.[32] The clinical utility of such a bank remains to be defined.

CELLULAR THERAPY TARGETING EBV+ LPD WITH EBV OF TYPE I AND II LATENCIES

In conditions other than organ allografting where severe immunosuppression is not involved, EBV is present in type I and II latencies. Although some EBV antigens are expressed, neoplastic cells might have evaded the innate host immune response. Adoptive transfer of a population of EBV-reactive cytotoxic T cells may potentially provide cellular control of the neoplastic cells.

In LPD with EBV of type I and II latencies, only a limited number of EBV antigens are expressed on the neoplastic cells. Therefore, the efficacy of EBV-specific T cells is not expected to be as good as for PTLD. Experiments *in vitro* had shown that cloning of T cells reactive to LMP1 and LMP2, which are generally expressed in type II latency in Hodgkin lymphoma, could be achieved.[33] The clinical feasibility of such an approach was subsequently demonstrated, where autologous EBV-specific T cells generated *ex vivo* from patients with EBV+ Hodgkin lymphoma were shown to persist to up to 13 weeks *in vivo*.[18] These observations were later confirmed in a larger number of patients,[34] suggesting that autologous EBV-specific T cells is potentially effective in selected patients with relapsed EBV+ Hodgkin lymphoma.

In chronic active EBV infection, which is another disease that typically involves EBV in type II latency, the infusion of autologous EBV-specific T cells led to an objective response in four of five patients.[35]

More recently, the above observations were replicated in patients with EBV+ lymphomas of various histopathologic

Table 2 Cellular therapies for Epstein Barr virus-associated lymphoproliferative diseases

Type of cellular therapy	*Disease*	*Advantages*	*Limitations*
DLI	PTLD after allogeneic HSCT	Available from most sibling donors Complicated manufacturing process not required	Development of GVHD Generally not available from MUD and UCB
Donor EBV-specific T cells	PTLD after allogeneic HSCT	Available from most sibling donors Not associated with GVHD	Generally available from MUD and UCB Complicated and time-consuming process of manufacturing EBV-specific cytotoxic T cells from individual donors
Autologous EBV-specific T cells	PTLD after solid organ transplantation	Not associated with graft rejection No issue with HLA matching	Complicated and time-consuming manufacturing process EBV-specific cytotoxic T cells Long-term persistence of EBV-specific cytotoxic T cells may not be achievable with continued immunosuppression
Third party EBV-specific cytotoxic T cells	PTLD after allogeneic HSCT, UCB HSCT and solid organ transplantation	Not associated with GVHD Less time-consuming if a bank of allogeneic EBV-transformed lymphoblastoid cell lines available	A bank of allogeneic EBV-transformed lymphoblastoid cell lines required HLA may not be fully matched
Autologous EBV-specific T cells	EBV-lymphoproliferative diseases with type II or III EBV latencies	Not associated with GVHD	Genetic engineering of EBV-transformed lymphoblastoid cell lines required

Abbreviations: DLI, donor lymphocyte infusion; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; MUD, matched unrelated donor; PTLD, post-transplantation lymphoproliferative diseases; UCB, umbilical cord blood.

subtypes.[36] In 20 patients with relapsed EBV+ lymphomas, complete responses were observed in 4 of 6 NK/T-cell lymphoma cases, 3 of 8 Hodgkin lymphoma cases, 2 of 4 cases of diffuse large B-cell lymphoma and 1 PTLD case. Because all of these lymphomas harbored type II latency EBV, the results indicate that EBV-specific T cells are also effective for LPDs not expressing highly immunogenic EBV antigens, as observed in malignancies involving type III latency EBV.

Although these results are exciting, they must be considered in the context of other targeted therapies for these lymphomas (Table 2). Furthermore, the complicated logistics of manufacturing EBV+ autologous T cells may limit the use of this form of treatment outside of clinical trials.

METHYLATION OF EBV GENOME AS A POTENTIAL THERAPEUTIC TARGET

In EBV-infected cells, the virus utilizes the expression of several different gene programs to control the expression of proteins on the infected cells. The regulation of expression is controlled by various epigenetic modifications to histone and DNA in the EBV genome.[37] Methylation of the CpG islands of the major EBV latency promoter occurs in circulating EBV+ memory B cells, leading to type I latency.[38] Similarly, CpG methylation of the EBV promoters also occurs in Burkitt lymphoma (type I latency) and Hodgkin lymphoma (type II latency).[39] These epigenetic alterations suppress the expression of highly immunogenic EBV proteins characteristically found in the viral lytic phase, so that EBV-infected cells are able to evade immunosurveillance mechanisms.[40]

Epigenetic changes in the EBV genome may also indirectly lead to epigenetic alterations of EBV-infected cells. EBNAs and LMPs interact with many proteins involved in controlling host cell DNA and histone modifications.[40] In neoplastic cells, these epigenetic changes repress the production of key tumor suppressors and contribute to the malignant phenotype.

The understanding of the epigenetic control of the latent-lytic switch in the EBV genome provides the theoretical framework of the 'lytic-induction therapy.'[40] In this approach, drugs that potentially reverse epigenetic changes in the EBV genome are postulated to induce the virus to undergo lytic phase proliferation in tumor cells, thereby leading to the expression of viral thymidine kinase. Therefore, when the antiviral drug ganciclovir is administered, it is phosphorylated by the viral thymidine kinase and subsequently becomes toxic to the neoplastic cell containing the lytic virus.[41] This concept had been tested in 15 patients with PTLD, B-cell, T-cell, NK-cell and Hodgkin lymphoma.[42] Ten patients showed significant antitumor responses, and four patients achieved complete remission.

Another interesting observation was that the chemotherapeutic drug cyclophosphamide appeared to induce viral lytic phase in endemic Burkitt lymphoma.[43] On the basis of this proposition, a phase I study recently evaluated the concomitant use of valacyclovir and cyclophosphamide in endemic Burkitt lymphoma and showed that this combination was safe.[44] Further clinical testing of this concept is needed.

Although these results appear promising, they must be compared with emerging novel and targeted therapies for these lymphomas.

ANTIVIRAL TREATMENT IN EBV+ LPD

Except when used together with epigenetically active agents,[41–44] antiviral drugs exhibit no direct effect on EBV+ LPD. However, when used prophylactically, antiviral drugs appear to suppress EBV replication and hence decrease the occurrence of PTLD after HSCT or solid organ allografting.[45–48] Antiviral drugs also appear to be effective in suppressing EBV replication in immunocompetent subjects.[49–51] Hence, in patients at high risk of EBV+ PTLD, the use of prophylactic antiviral drugs may be warranted.

CONCLUSIONS

In EBV+ LPD, EBV is an attractive therapeutic target. Cellular therapy targeting EBV is an important validation of the efficacy of the immune system against neoplastic cells. However, these strategies must be compared with the emerging availability of gene- or pathway-targeted therapies. Finally, the production of EBV-specific T cells must be streamlined to attain a more timely and affordable treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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