Chapter

Epstein-Barr Virus: Should We Still Invest in Vaccines or Focus on Predictive Tests?

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Abstract

The complex interplay between host and EBV has made it difficult to elaborate useful vaccines protecting against EBV diseases. It is encouraging to see that EBV vaccine programs have started to incorporate different arms of the immune system. An array of argument calls for a realistic goal for vaccine strategies which should be preventing EBV diseases, rather than EBV infection. EBV is the primary cause of infectious mononucleosis and is associated with epithelial cell carcinomas, as well as lymphoid malignancies. Parallel to this need, one could propose priorities for future research: (i) identification of surrogate predictive markers for the development of EBV diseases (ii) determination of immune correlates of protection in animal models and humans.

Keywords: vaccine, early diagnosis, lymphomas, EBV diseases

1. Introduction

More than 95% of the world's adult population is infected with the Epstein–Barr Virus (EBV or HHV4), a virus belonging to the *Herpesviridae* family that mainly infects B lymphocytes. Human Herpesviruses (HHV1–8) have co-evolved through persistent infections in their hosts which are spread efficiently to others and generally do not cause serious disease (**Table 1**) [1]. EBV is transmitted by saliva, usually infects its host during infancy and is largely asymptomatic. If the infection does occur later, in adolescence or young adulthood, in about 40% of cases it leads to the development of an acute condition called infectious mononucleosis (IM). In the United States alone, 125,000 cases of IM are reported each year. EBV is also associated with the development of several malignancies derived either from lymphocytes or from epithelial cells (**Table 1**). It is estimated that about 10% of cancers associated with a viral infection are associated with EBV and that each year, on average, about 200,000 new cases of EBV-associated cancers are diagnosed worldwide [2]. Furthermore, EBV is also associated with the development of autoimmune diseases such as multiple sclerosis [3]. The complex interplay between the Herpesviruses and their hosts has made it difficult to elaborate useful vaccine strategies to protect against HHV-associated diseases [4]. Over the years, the development of HHV vaccines has been a story of mixed fortunes, especially for HSV-2 and HCMV (**Table 2**). The frequent presence of EBV in many pathologies is an indicator of the necessity of developing a vaccine against EBV. The argument was first put forward

Subfamily Herpesviridae	Common abbreviation	Common name	Common manifestations	Antiviral therapy
Alpha-	HSV-1	Herpes Simplex Virus type 1	Cold sores, keratitis, encephalitis	+++
Alpha-	HSV-2	Herpes Simplex Virus type 2	Genital sores	+++
Alpha-	VZV	Varicella Zoster Virus	Chicken pox, shingles	+++
Beta-	HCMV	Human Cytomegalovirus	Severe diseases in the immunocompromised host	++
Beta-	HHV-6	Human Herpesvirus-6	Roseola infantum, rash & fever	—
Beta-	HHV-7	Human Herpesvirus-7	Roseola infantum, rash & fever	—
Gamma-	EBV	Epstein-Barr Virus	Infectious mononucleosis, lymphoid malignancies, nasopharyngeal & gastric carcinoma	+/–
Gamma-	HHV-8	Human Herpesvirus-8	Kaposi sarcoma	—

+++ widely used and successful, ++ widely used and quite successful, + occasionally used with limited success, – rarely used with an uncertain outcome.

Table 1.
List of the major herpesviruses pathogenic for humans. First the alpha- including neurotropic viruses, second the beta- with the most salient virus, CMV. This virus infects a large number of cells and is responsible for a lot of serious diseases in the immunocompromised hosts. HHV6 and HHV7 are lymphotropic viruses, responsible of roseola, and rash and fever in adults. Finally, the gamma- which includes B lymphotropic viruses with transforming activities.

more than 40 years ago by Sir Antony Epstein, the pathologist and expert electron microscopist who discovered the EBV virus [5]. However, to date, despite sustained efforts, the EBV vaccine has not been finalized, even though promising results have been obtained [6, 7]. The main difficulty in developing an anti-EBV vaccine stems from the patchy nature of our knowledge of the course of EBV infection *in vivo.*

Below we review the history of the EBV vaccine development, and current strategies involved. At the same time, one could propose priorities in terms of future research, such as (i) a better definition of the goal for an EBV vaccine; and (ii) the identification of costless surrogate markers that predict the development of EBV-related malignancies.

2. The first challenge for EBV vaccines: the complexity of the biological cycle of EBV

The EBV lifecycle is considerably complex and passes through a phase of latent infection during which the virus induces the activation, proliferation, and differentiation of primary B cells into memory B cells [8, 9]. During this phase, the infection elicits a humoral and cellular immune response directed against the proteins of the latent phase. During the terminal differentiation into plasma cells of infected cells, the productive viral cycle is activated and virions are produced which will be able to infect epithelial cells capable of producing a large number of viral particles. The numerous viral proteins expressed during the production cycle are also important targets of the cellular immune response [10]. The EBV encodes

Herpesvirus	Site of latency and persistence	Pathology	Vaccine trials	Antivirals	Prevalence	Transmission
HHV1 (HSV1)	Neurons (sensory ganglia)	Widespread vesicular lesions and neurological diseases	No ongoing vaccine research	YES	High	Skin contact
HHV2 (HSV2)	Neurons (sensory ganglia)	One of the most prevalent sexually transmitted infections worldwide	In clinical trials no regulatory-approved vaccines	YES	High	Sexual
HHV3 (VZV)	Neurons (sensory ganglia)	Chickenpox	Live vaccine available	YES	High	Respiratory tract
HHV4 (EBV)	B Lymphocytes (oropharyngeal epithelium)	IM, lymphoid & epithelial tumors	In clinical trials no regulatory-approved vaccines	YES	Very high	Saliva
HHV5 (CMV)	Blood monocytes /bone marrow precursors (probably epithelial cells)	Significant disease in pregnancy and immunocompromised patients	In clinical trials no regulatory-approved vaccines	YES	High	Sexual, blood, saliva, urine
HHV6	Monocytes, T lymphocytes	Roseola infantum	—	Not relevant		
HHV7	Monocytes, T lymphocytes	Roseola infantum	—	Not relevant		
HHV8 (KSHV)	Uncertain	Kaposi's sarcoma	No ongoing vaccine research	Not relevant	Moderate	Sexual

Table 2.
Main features of the HHVs. Some vaccines exist, for example the Varilrix© and Zostavax© against VZV. Clinical studies about some other vaccines are in progress (CMV and EBV).

approximately 80 proteins, 15 of which possess at least 90 antigenic epitopes. A large quantity of these proteins stimulates the T-cell receptors (TCRs), but a few interact with the B-cell receptors (BCRs) [11]. Activation of B cells and subsequent antibody production has not only been related to at least 3 envelope glycoproteins (mostly gp350) but also to latency-associated membrane proteins (LMPs). The majority of EBV epitopes inducing either cytotoxic and/or helper T lymphocytes were located on non-structural and/or latency associated proteins. In acute IM patients (approximately 40%), a considerable proportion of HLA B8 restricted CTL reactivity is directed against a single peptide (RAKFKQLL) of the *trans*-activator protein BZLF1/Zta/ZEBRA [10].

It must be noted that natural killer cells and anti-EBV cytotoxic T lymphocytes (CTLs) are the main players in the immune response that effectively controls infection [12]. The primary role of anti-EBV CTLs would be to control the proliferation

Vaccine candidates	EBV antigens used	Results
Epitope vaccine	EBNA3A	Induced EBNA3A-specific T-cell responses Did not protect against EBV infection. Lower incidence of IM in vaccinated people
Antigen–antibody conjugates	EBNA1 Several latent antigens	Targeting of DC enabled the induction of EBNA-1spcefic CD4+ and CD8+ T cells Vaccination of humanized mice generated EBNA1 specific T cells
Monomeric	gp350	Induced neutralizing gp350-specific antibodies. Reduced incidence of IM Did not protect against EBV infection.
Multimeric	Tetrameric gp350 Trimeric gH/gL and trimeric gB	Higher immunity (neutralizing antibodies) of tetrameric gp350 compared to that of monomeric one (vaccinated rabbits). Higher immunity (neutralizing antibodies) of trimeric gH/gL and trimeric gB compared to that of monomeric gp350 (vaccinated rabbits).
Nanoparticles	gp350	Higher immunity (neutralizing antibodies) of gp350-containing nanoparticles, compared to that of monomeric gp350 (vaccinated mice and monkeys).
Chimeric NDV VLPs	gp350 gH/gL, gp42, LMP2 & EBNA1	Higher immunity (neutralizing antibodies) of gp350-containing NDV*-VLPs, compared to that of monomeric gp350 (vaccinated mice). Use of NDV*-VLP platform to combine EBV lytic and latent antigens
EBV VLPs	More than three dozen structural proteins More than three dozen structural proteins & EBNA1	Similar antigenicity with wt EBV Increased protection against EBV infection (humanized mice)
Recombinant adenovirus	ZEBRA immediate-early protein (BZLF1 gene)	Prolonged survival from fatal EBV-LPD (humanized mice)
mRNA	mRNA-1189 (gp350-gH/gL-gB) Moderna Inc. platform	Antibody titers against viral proteins involved in epithelial cell entry (gH/gLand gB) or B cell entry (gp350, gH/gLand gB) were measured in peripheral blood at day 57 (mice)

Table 3.
*Summary of prophylactic EBV vaccine candidates that have been developed (from ref. [7]) * NDV Newcastle disease.*

of latently infected B cells. EBV has the feature of implementing various latency and lytic transcription programs, suggesting that it assumes distinct antigenic states within infected individuals (**Table 3**) [7, 10]. Yet, despite the wide variety of antigens that predominate throughout the EBV life cycle, EBV vaccine candidates have traditionally only focused on a limited number of EBV antigens. For a summary on these vaccine candidates see a review by Cohen [13]. Thus, the EBV vaccines designed so far fall into two categories: those preventing any kind of infection (including prophylaxis of EBV-associated malignancies) and those designed for therapeutic purposes (to be used in subjects already infected) [13].

3. A second challenge: the lack of a true animal model

EBV is highly species-specific and only infects humans and primates. Initial studies of EBV vaccines used cottontop tamarins (white-crested), a now endangered species [14]. This model has several drawbacks including the very high doses of EBV in the challenge inoculum required to cause tumors, a non-physiologic route of infection (intraperitoneal injection of virus), and the fact that EBV is not a natural pathogen in these animals. Moreover, EBV does not establish a latent infection in the B cells of these monkeys which is the case with humans. EBV vaccine studies have also been performed in common marmosets and EBV gp350 can protect against the parenteral challenge of these animals [15]. The use of an animal virus such as *Rhesus* lymphocryptovirus (LCV) is also a useful model for vaccination studies [16] as it is close to EBV and reproduces the majority of EBV symptoms in its natural host (the *Rhesus* macaque). Today, the humanized mouse model looks promising, as it is possible to recreate different pathologies associated with EBV [17–19]. However, the absence of infection in the epithelial cells of the animal does not allow the reconstitution of all the pathologies associated with EBV in humans. Two types of vaccines are currently being studied to control EBV: A prophylactic vaccine that aims to neutralize the virus to block infection and a therapeutic vaccine that aims to induce or improve the anti-EBV cellular response in some patients.

4. The prophylactic vaccines

In designing a prophylactic vaccine against a virus, induction of a neutralizing antibody response is generally sought. Multiple alternative vaccine candidates include targeting EBV-based glycoproteins, EBV lytic proteins, and EBV latent proteins (**Figure 1**).

4.1 Which antigens are used?

The glycoproteins of the viral envelope are therefore the preferred targets. EBV carries several glycoproteins (gp350, gB, gH, gL, gp42, gM, gN, BMRF2, BDLF2, BDLF3, BILF2, BILF1, BARF1) [20] on its surface. To date, the greatest strides towards developing an EBV-based vaccine have been made by targeting gp350. This type I glycoprotein is crucial for EBV's ability to enter the host B cells by binding their CD21 or CD35 receptor. It is the most abundant glycoprotein on the surface of virions and the most expressed by cells infected with EBV. In addition, gp350 is the major target of antibodies capable of neutralizing infection of B cells [20]; it is also an important antigen and target recognized by cellular immunity [10]. Other EBV glycoproteins such as (i) gH/gL (member of the fusion complex); (ii) gp42 (determines the cellular tropism of EBV); and (iii) gB (a class III type of viral fusion protein

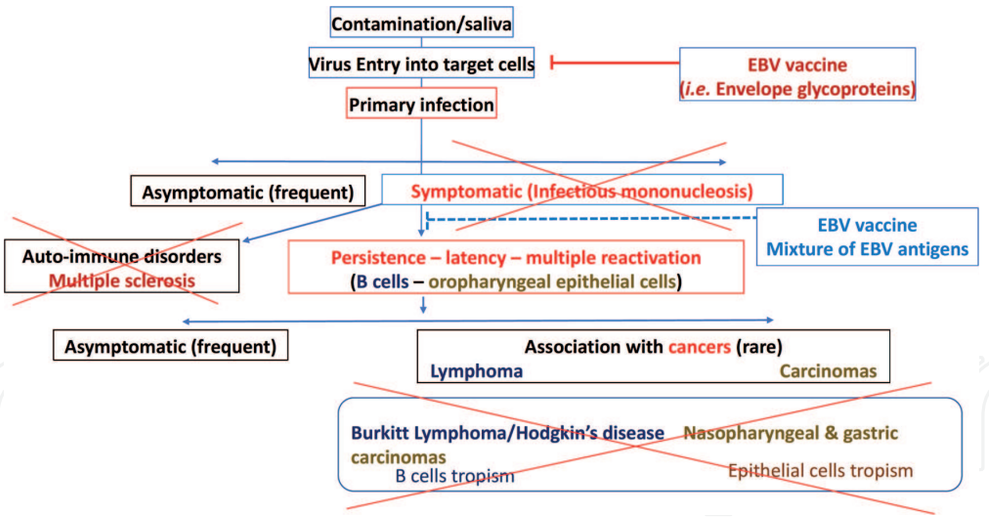

Figure 1.
An EBV vaccination strategy: Whatever EBV vaccine candidates, they will not block infection. However, they could prevent the onset of the symptoms of infection and reduce the risk of developing EBV-associated tumors or EBV-associated pathology (i.e. MS).

also essential for maturation and egress) have also been tested in various vaccine trials [7]. They are also able to induce the expression of neutralizing antibodies. They do, however, appear to be less effective than those directed against gp350 (see **Table 3**).

For the various reasons cited above, most vaccination trials have been carried out using recombinant gp350 [6, 7, 13]. The production of viral-like particles (VLPs) which do not contain a genome and in which several viral proteins involved in cell transformation have been inactivated or deleted has also been described. The vaccination of mice with these VLPs induces the production of neutralizing antibodies and cellular immune response [7, 21]. Although, while this approach is promising, given the oncogenic properties of EBV, the acceptability of this type of vaccine for use in human health remains uncertain.

4.2 The clinical trials

Several vaccines have been evaluated to prevent infection and protect against the symptomatic episode of primary infection (IM) [13]. The first vaccine trial in humans was undertaken by Gu and colleagues [22] using a live recombinant vaccinia virus/major EBV envelope antigen BNLF-1 MA (gp220–340) construct. The authors showed that for the first time it was possible to protect against and/or delay EBV infection by the natural route. The most advanced study of the safety, immunogenicity and efficacy of an EBV vaccine has been reported by Sokal and colleagues [23]. In their study (NCT00430534), a group of 181 EBV seronegative volunteers between the ages of 16 and 25 received three doses of a recombinant gp350 vaccine or placebo. The authors claimed the vaccine had demonstrable efficacy (mean efficacy rate, 78.0% [95% confidence interval: 1.0–96%]) and that there were no concerns regarding safety or immunogenicity. Over an observation period of 18 months, the vaccination of the young adults with recombinant gp350 reduced the risk of developing an IM of 10% in controls to only 2% in vaccinated people. However, despite the production of neutralizing antibodies, vaccination does not appear to prevent infection. This result suggests that the vaccine used may reduce the risk of associated pathogenesis without necessarily preventing infection. The same type of vaccine that was given to patients not infected with EBV, and who were waiting for a kidney transplant, did not seem to give satisfactory results (only 30% produced neutralizing antibodies). The unsatisfactory results were probably

because patients in this study suffered from both an immunosuppressed state and renal impairment [24].

Alternative EBV vaccines such as, a virus-like particle EBV vaccine [21] and a CD8+ T-cell peptide epitope-based vaccine [25]. have been evaluated in Phase 1 clinical trials. To date, several phases I and phase II clinical trials have been carried out and have yielded rather encouraging results [7, 26].

In 10% of cases, IM is associated with quite severe symptoms (such as prolonged asthenia, risk of Hodgkin's lymphoma) [27–29] and in the most serious cases with neurological involvement (1%) [3, 30]. Given the vast variability in these results and the long period from an EBV infection to the onset of MS, such EBV vaccine trials in MS populations are not feasible. With a 30 times greater rate of MS occurrence in first-degree relatives when compared to unrelated populations, such intervention may potentially decrease the overall MS incidence [31]. This explains the increasing interest in developing EBV vaccines to prevent MS. Given the association of IM with MS, there is a strong possibility of reducing this childhood infection by eradicating MS. [32]. So far, however, there is no licensed EBV vaccine and to make progress regarding its development, a greater understanding of the association of EBV with MS is required [33]. Recent advances have pointed to the use of EBV vaccines to control MS. Indeed, both asymptomatic EBV infection and IM have also been associated with an increase in MS susceptibility [33, 34]. The imminent increase in MS risk following an EBV seroconversion has been expertly shown through a study that utilized serial blood samples derived from more than 8 million active-duty military personnel [35].

Other potential targets for vaccine development include immediate and early EBV proteins that are expressed during the first steps of the lytic cycle. Both Zta and Rta immediate proteins (encoded by BZLF1 and BRLF1, respectively) are easily recognizable due to an uninhibited CD8+ T-cell response [36]. On the other hand, the early lytic proteins BMLF1 and BMRF1 can be detected by CD4+ T cells as early as the first day of EBV infection [37]. Studies have examined the utility of the BZLF1 vaccine in mice models of EBV-induced post-transplant lymphoproliferative disorder and shown successful T-cell immunity induction towards the infected tumor cells [38]. Lastly, recent evidence also shows that the latent proteins (EBNA) can be recognized by CD8+ and CD4+ T cells and prevent further expansion of EBV-infected B cells [37]. As we now understand the importance of B cells in the MS pathophysiology, we can conclude that this type of vaccine intervention would potentially exert a therapeutic outcome [39]. In contrast, an effective EBV vaccine that could prevent the 200,000 new EBV-associated malignancies occurring globally each year is not currently available despite the considerable efforts expended in developing EBV gp350 vaccines [6]. Very recently, in 2020, the Moderna Company (Cambridge, MA, USA) carried an innovative mRNA-based EBV vaccine (mRNA-1189) (https://investors.modernatx.com/static-files/834b6509-553f-4ee5-84e0-c198bbb850f0). Preclinical data demonstrated the ability to induce antibodies against EBV antigens: Naïve Balb/c mice were given two doses of a vaccine against EBV antigens in combination approximately 4 weeks apart. Antibody titers against viral proteins involved in epithelial cell entry (gH/gL and gB) or B cell entry (gp350, gH/gL and gB) (**Table 3**) were measured in peripheral blood at day 57. Their last results demonstrated high levels of anti-EBV neutralizing antibodies, and at levels significantly higher than those observed in naturally-infected human sera.

4.3 Major drawbacks of the prophylactic EBV vaccine strategies

Contrary to Epstein's initial idea, an EBV vaccination does not block infection. However, it could prevent the onset of the symptoms of infection and reduce the

risk of developing EBV-associated tumors. Moreover, the correlates of protection against EBV infection and diseases (in animal models and humans) are not clearly defined, so it is hard to reliably predict the ideal EBV vaccine targets and whether humoral immunity or cellular immunity or both should be involved. Currently, the definitions of a goal for an EBV vaccine and criteria for licensure to prevent diseases rather than infections are not clear.

5. The therapeutic vaccines

In the case of a therapeutic vaccine, the induction of cellular immunity against EBV in patients suffering from certain pathologies (NPC, HD, etc.) is the main objective. One of the difficulties of this approach is that the number of viral proteins expressed in cancer cells varies according to the pathology concerned. The EBNA-1 protein is the only viral protein expressed in all cases of EBV-associated cancers. It is also one of the main targets of CD4 T cells along with the membrane proteins LMP1 and LMP2 which are also good targets for CD8 T cells. This makes this type of approach possible. The relevance of designing a therapeutic anti-EBV vaccine is based on clinical observations from tests of infusions of EBV-specific T lymphocytes (CTLs directed against the viral proteins LMP1 and LMP2). In patients with Hodgkin's lymphoma, non-Hodgkin's lymphoma or nasopharyngeal carcinoma (NPC), the results of the first studies are encouraging - this specific EBV cell therapy can markedly improve the survival of some of these patients [7, 40–42]. Therefore, a vaccine that induces T-cell responses to tumor-expressed EBV latency proteins could improve patient survival.

In the context of cure therapy, the adoptive transfer of EBV-specific T cells has been therapeutically explored for decades with clinical success [43]. To avoid naturally occurring EBV-specific autologous T-cell selection from every patient, the transgenic expression of latent and early lytic viral antigen-specific TCRs essential to redirect T cells and to target the respective tumors has been investigated. The latest evidence suggests that not only TCRs against transforming latent EBV antigens, but also against early lytic viral gene products, might be protective for the control of EBV infection and associated oncogenesis [44]. At the same time, these approaches might be more selective and cause less collateral damage rather than targeting general B-cell markers with chimeric antigen receptors (CARs). Thus, EBV-specific TCR transgenic T cells constitute a brilliant therapeutic strategy against EBV-associated malignancies [45]. As an example, recent studies describing $CD8^{+}$gp350CAR-T cells showed proof-of-concept preclinical efficacy against impending EBV^{+} lymphoproliferation and lymphomagenesis [46].

6. Uncertainties surrounding EBV vaccines

Despite the very encouraging results obtained in phase II clinical trials, to date, no phase III trials have been implemented. The reason why no further development of this vaccine has been done is not known. Given the large diversity of pathologies associated with EBV, it is unlikely that a single vaccine applicable to all diseases associated with EBV can be developed. Vaccination against EBV must take into account various factors such as the geographic characteristics of certain pathologies (NPC in South-East Asia, endemic Burkitt's lymphoma (BL) in equatorial Africa), the incubation period necessary between infection and development of the disease pathology (IM: 4 to 6 weeks, NPC: > 30 years), and the initial age of infection. Such

disparity complicates vaccination strategies which would need to be implemented. Despite this and depending on the pathology involved, it is still worth considering further research on an anti-EBV vaccination program. According to a recent US study among university students, 37% were EBV negative when they entered university, but 3 years later 46% of them had experienced EBV seroconversion. Of these, 77% went on to develop an NID. It is interesting to note that IM is the most common cause of absenteeism among new recruits to the US military. In developed countries, these epidemiological observations support the idea of administering a vaccine capable of preventing the disease when administered to children aged between 11 and 12 who are EBV negative (in tandem with the administration of a vaccine such as a papillomavirus). Such a vaccine would reduce the risk of developing IM and would also reduce the risk of developing Hodgkin lymphoma [47] or MS [30], pathologies which are linked to EBV and which are most likely to be a consequence of the EBV-induced immunological disorder in IM [48].

The value of an EBV vaccination to protect children against BL, especially in Africa, is certainly significant. Nevertheless, in this region of the world, infection with EBV generally occurs early (50% of children are infected by their 1st year), and it is certainly not easy to vaccinate at this stage, especially if three injections are required to achieve protective immunity. It is, however, not impossible and has been implemented in some countries where children are given vaccinations which include hepatitis B in early childhood.

EBV is associated with various lympho-proliferations in immunocompromised people, especially following transplantation, or HIV infection. The risk of developing PTLD is 25 to 30 times higher in an EBV-negative person before the transplant than in a person who was HIV-positive. Prophylactic vaccination against EBV would not only reduce the risks associated with the primary infection but could also decrease the risk of developing PTLD during transplantation; the latter hypothesis has not yet been evaluated. Regarding NPC and gastric carcinoma, only retrospective studies after prophylactic vaccination could reveal its efficacy. It would then be necessary to demonstrate the direct effects of a prophylactic vaccine aimed at preventing these pathologies which develop more than 30 years after infection. Nevertheless, this has already been achieved with the hepatitis B vaccination program which is performed in children and protects against the development of liver cancer 15 to 20 years later.

7. The EBV diagnostic tests as a predictor of diseases

Taking into account the above-mentioned points, it is undoubtedly time to turn to predictive tests to prevent the appearance of the first signs of pathology both in the context of cancers (lymphomas) and in the context of chronic pathologies.

7.1 What about conventional EBV diagnostic tests?

EBV serology was for a long time the only technique used for diagnosis. In immunocompromised patients, serological tests (looking for IgG and IgM antibodies directed against two types of viral antigens - capsid and EBNAs) are used to identify the immune status to EBV in the donor/recipient (before transplantation) and in HIV patients. They are not used for primary or hereditary immunodeficiencies. With regards to EBV serology, the practice is relatively homogeneous with an assay combining anti-VCA IgG, anti-VCA IgM and anti-EBNA IgG. The combined use of these three markers is sufficient for most diagnoses, making it possible to distinguish

the primary infection (±, +, −) from an old infection (+, −, +). Depending on the manufacturers of diagnostic kits, the detection technique (ELISA or immunofluorescence) and the nature of the antigen targets (recombinant proteins, infected cells, peptides, etc.) of these antibodies may vary, but most of the techniques used are validated for their diagnostic capacities by expert medical virology laboratories at the time of marketing.

The ability to accurately determine viral load (DNA PCR) for HHV infections post-transplantation [4] has become a mainstay for diagnostics especially in the context of the beta and gamma herpesviruses. Most approaches use real-time quantitative PCR-based assays [49]. PTLD (classified into six categories by WHO) has become a deleterious complication of both solid-organ and hematopoietic stem-cell transplantation. Data from large transplantation registries have shown an increased incidence of PTLD and significant associations with morbidity and mortality [50, 51]. EBV viral load monitoring is now routine and high viral loads are often associated with concurrent PTLD. But data linking EBV kinetics to the risk of developing PTLD remain controversial. Measurement of EBV viral load by quantitative PCR is an essential test in the follow-up of children with solid organ transplants. It is used: (i) to prevent the development of PTLD (by adapting immunosuppression and/or by initiating pre-emptive treatment); (ii) in the monitoring of pre-emptive treatment; and (iii) in the follow-up of the curative treatment of PTLD.

It should be emphasized that there is a difference between patients who have had solid organ allografting and HSCT patients [50]. Immunosuppressive treatments in solid organ allograft recipients are modest compared to HSCT recipients who receive more severe immunosuppressive treatment. Correlations between higher EBV loads and the development of PTLD are seen in solid organ allograft recipients, but these correlations do not indicate high positive and negative predictive values [52]. There is considerable overlap between the EBV loads in patients with PTLD and those in patients without PTLD. Furthermore, solid organ allograft recipients receive lifelong immunosuppression so that there is a long-term risk of EBV-PTLD. Therefore, routine surveillance for EBV-DNA by quantitative PCR is not recommended in adult recipients [53]. Solid-organ allograft recipients also sometimes carry chronic high EBV loads without symptoms consistent with PTLD [53, 54]. However, the significance of a high EBV load in terms of long-term health is unknown. Conversely, in children at high risk of primary EBV infection, routine surveillance is beneficial for the preemptive identification of patients at high risk of PTLD [53]. Finally, a current article investigating both the EBV DNA load in whole blood and EBV serology in HIV-infected patients with classical Hodgkin concluded that EBV DNA loads at diagnosis were not prognostic [55].

Not unlike the situation with CMV, the lack of an international genome standard for quantification of EBV in molecular assays makes a comparison of thresholds for impending PTLD difficult to interpret [4]. In contrast, EBV infection and in particular EBV-driven PTLD is a more difficult disease to manage with little evidence that antiherpes drugs are effective especially once PTLD is manifest [4]. Anti-CD20 antibodies (Mabthera®) are not introduced until PTLD has been confirmed. In contrast, for other types of transplants (intestines, lung, heart, kidneys), anti-CD20 antibodies can be used earlier and they are part of the pre-emptive treatment. There is currently no consensus on the best preemptive strategy because the threshold, or kinetics, of preemptive intervention is difficult to define. Typically, a one-log increase in viral load is a warning sign. It should be noted that the therapeutic decision is based on a set of arguments which are virological, clinical (such as tonsillar

hypertrophy for example) or biological (for example LDH, uric acid)), because the EBV viral load is not the only predictive marker of PTLD.

7.2 New biomarkers and therapies exploring the lytic cycle

For decades, many articles have reported the presence of an EBV lytic cycle in tumor cells from HL, NPC, in transplant patients, and breast tumors. Clinical studies on EBV lytic proteins including ZEBRA in patients with PTLD or HIV-associated non-Hodgkin lymphoma NHL are mostly related to the role of these proteins in neoplastic tissues. Both high EBV copy number and strong BZLF1 mRNA expression in the peripheral blood lymphocytes (PBL) of patients are sensitive markers of EBV-related PTLD. Soluble ZEBRA concentrations of >100 ng/mL detected by an enzyme-linked immunosorbent assay (ELISA) in serum of patients after solid organ or hematopoietic stem cell transplant were predictive of PTLD in 80% of the cases within 3 weeks [56] (for a review see ref. 57). Thus, ZEBRA testing in serum could help identify patients likely to develop severe outcomes during the critical post-transplant period and serve as a potential diagnostic/prognostic marker for EBV follow-up in immunocompromised patients (**Figure 2**).

The relevance of the EBV lytic cycle to human pathology prompted researchers to target certain lytic proteins with therapeutic aims [57]. As an example, adenovirus vectors expressing BZLF1 or BRLF1 were used to treat EBV-positive tumors [58]. On the other hand, the Food and Drug Administration (FDA)-approved leflunomide, which targets EBV replication, was shown to inhibit the earliest step of lytic EBV reactivation (BZLF1 and BMRF1 expression) and prevented the development of EBV-induced lymphomas in both a humanized mouse model and a xenograft model [59]. More recently, duvelisib (a molecule inhibiting the PI3K/AKT signaling pathway, and BCR signaling) was shown to reduce cell growth and expression of EBV lytic genes BZLF1 and gp350/220 in EBV-positive cell lines [60]. The histone deacetylase (HDAC) and DNA methyltransferase inhibitors are also possible avenues to suppress the ZEBRA expression and the entire lytic cascade [61]. To summarize, efforts should be made to improve the relevance of using ZEBRA protein in future EBV vaccine settings [62].

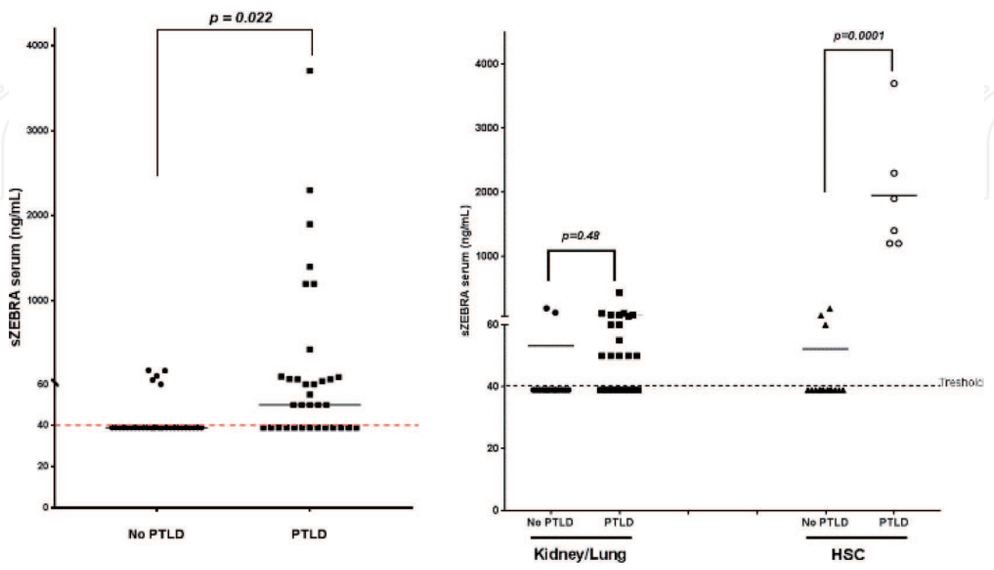

Figure 2.
ZEBRA as a specific marker measuring early activation of replication of the oncogenic EBV, providing more precise monitoring of posttransplant lymphoproliferative disorder development in transplant patients. (from Habib et al. [56] with permission) (HSC = hematopoietic stem cells).

8. Conclusion

The issue of an EBV vaccine is a very actual topics since there is more and more evidence for an association between EBV primary infection (IM) and the development of multiple sclerosis (MS) and Hodgkin's lymphoma. Numerous prophylactic IM vaccines targeting EBV proteins have been developed. They have shown partial success in reducing IM but have failed to prevent EBV infection. Therapeutic vaccines against NPCs have had considerable success, but there is a need to improve their effectiveness. Increasing vaccine activity in NPC (or gastric carcinoma) might be difficult due to a long latency period between primary infection and the development of these carcinomas (see **Table 4**) [63]. The addition of new targets and the recent advances in mRNA vaccines may further improve the efficacy of therapeutic and prophylactic vaccines against EBV [64].

However, the design of a prophylactic vaccine against EBV poses serious problems: It is still difficult to find exact correlates of protection and it is still problematic to define the populations intended to receive the vaccine. Immunotherapeutic strategies, including CAR T cells, are emerging as new platforms for the treatment of tumors associated with EBV [45, 46]. The incorporation of immunotherapeutic strategies as first-line treatment may provide better long-term results. It remains to be seen how the various immunotherapeutic strategies will be incorporated into future therapeutic strategies.

On the other hand, the design of new predictive tests (*i.e.* ZEBRA-based) capable of monitoring the intensity of EBV reactivation and tumor progression, could more easily help the physician to monitor the course of pathologies linked to

Prospects	Progress	Problems
Prevention of infectious mononucleosis	IM was prevented in a phase 2 study with a subunit gp350 vaccine [23] A CD8+ T-cell peptide (EBNA3-TT) vaccine was immunogenic with a hint of efficacy [25]	Duration of protection unknown. Viral loads and T-cell-specific responses were not evaluated. The ideal age' which to vaccinate may differ according to race/ethnicity and socioeconomics
Prevention of Nasopharyngeal Carcinoma	Vaccinia constructs expressing EBV glycoprotein (gp 220–340) are immunogenic and may have reduced incidence of EBV infection in Chinese children	CD8+ T-cell peptide vaccine: HLA restricted. The long incubation period from EBV infection to the development of NPC makes efficacy trials impractical.
Prevention of lymphomas	Subunit gp 350 vaccines are safe in pediatric renal transplant candidates	The vaccine was poorly immunogenic probably due to the low dose and weak adjuvant; the trial could not assess protection from PTLD
Treatment of NPC	Vaccinia recombinant vectors expressing the tumor-associated latent or lytic viral antigens aresafe and immunogenic [41, 42, 58]	Therapeutic efficacy has not yet been assessed
Prevention of multiple sclerosis	Evidence that a vaccine could work: EBV-specific CD8+ T cell responses are elevated during active MS [39].	The long incubation period from EBV infection to MS makes vaccine efficacy trials impractical except perhaps in first-degree relatives

Table 4.
Prospects, progress and problems in EBV vaccine development (from Balfour HH [63]).

EBV replication (*i.e.* lymphomas, MS). Such approaches will easily make it possible to initiate pre-emptive antiviral treatments; in addition, these diagnostic tests have the advantage of being minimally invasive and inexpensive.

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