Published in final edited form as:

Curr Opin Virol. 2014 June ; 0: 1–5. doi:10.1016/j.coviro.2014.02.005.

Progress, Prospects, and Problems in Epstein-Barr Virus Vaccine Development

Henry H. Balfour Jr., M.D.^{1,2}

¹Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis MN 55455

²Department of Pediatrics, University of Minnesota Medical School, Minneapolis MN 55455

Abstract

Epstein-Barr virus (EBV) is responsible for a farrago of acute and chronic human diseases including cancer. A prophylactic vaccine could reduce this disease burden. Several EBV vaccines have been given to humans but none has been sufficiently studied to establish safety and efficacy. EBV vaccine development has been hampered by the lack of an animal model other than subhuman primates, proprietary issues, selection of an appropriate adjuvant, and failure to reach consensus on what an EBV vaccine could or should actually achieve. A recent conference at the U.S. National Institutes of Health emphasizing the global importance of EBV vaccine and advocating a phase 3 trial to prevent infectious mononucleosis should encourage research that could eventually lead to its licensure.

Keywords

Epstein-Barr virus (EBV); EBV vaccine; infectious mononucleosis; EBV-associated cancers; multiple sclerosis

Introduction

Epstein-Barr virus (EBV) is an important global human pathogen. At least 90% of adults worldwide have been infected. The consequences of this are underappreciated. Primary EBV infection is responsible for most cases of infectious mononucleosis, which often results in significant loss of time from school or work in developed countries. EBV is the cause of several cancers including endemic Burkitt lymphoma, nasopharyngeal carcinoma, a subset of Hodgkin lymphomas, gastric carcinoma, lymphomas in HIV-infected individuals, and

© 2014 Elsevier B.V. All rights reserved.

Correspondence: Henry H. Balfour, Jr., M.D., University of Minnesota, Department of Laboratory Medicine and Pathology, MMC 437 Mayo, 420 Delaware Street, Minneapolis, MN 55455 (balf0001@umn.edu) Phone: 612-625-3998 Fax: 612-626-1923, Website: <http://cyp.umn.edu/>.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

posttransplant lymphoproliferative disorder (PTLD). EBV is also implicated as an important environmental risk factor for autoimmune diseases, especially multiple sclerosis (MS).

Development of a prophylactic vaccine, in my opinion, is the most important future step toward reducing the burden of EBV-associated diseases. Progress in this area has been painfully slow for various reasons including of a lack of an animal model except subhuman primates, proprietary issues, selection of an appropriate dose and adjuvant, and debate about what an EBV vaccine could or should actually achieve.

The first concrete EBV vaccine proposal was made by Epstein in 1976 [1]. He advocated the use of EBV-determined membrane antigen as immunogen, the suitability and need for cottontop tamarins as test animals, and assessment vaccine efficacy in humans by protection of adolescents from infectious mononucleosis. He also pointed out that the ability of a vaccine to prevent an EBV-associated human cancer could likewise be determined relatively quickly in a high incidence area for Burkitt lymphoma. Fast forward: more than three decades later we still do not have a viable EBV vaccine. Yet, participants attending a February 2011 meeting at the U.S. National Institutes of Health on EBV vaccine research recommended conducting clinical trials of an EBV vaccine to prevent infectious mononucleosis and EBV-associated cancers[2]. This disconnect between prospect and progress is disconcerting.

Progress: Prophylactic EBV vaccines

A summary of prospects, progress and problems in EBV vaccine development is provided in Table 1. Three prophylactic EBV vaccines have been evaluated in placebo-controlled clinical trials. Two vaccines were constructed to induce neutralizing antibody and one was designed to control expansion of EBV infected B cells by generating CD8⁺ T-cell immunity to EBV nuclear antigens (EBNAs).

Gu et al. performed a phase 1 vaccine trial in Beijing, China using vaccinia virus constructs expressing the EBV membrane glycoprotein gp220–350 [3]. After the vaccine was shown to be safe in 11 adults and 6 children who were latently infected by EBV, 19 EBV-naïve children 1 to 3 years of age were studied. Nine received the vaccine by scarification as a single dose containing 10⁷ pfu/mL of the recombinant vaccinia virus and 10 subjects served as controls. The vaccine was immunogenic and during 16 months of follow-up, 3 of 9 vaccinees and 10 of 10 in the control group became infected with EBV evidenced by development of antibodies against EBV viral capsid antigen. The authors concluded: “it has been shown for the first time that protection against and/or delay of EBV infection by the natural route is possible in humans.” No further work has been reported for this vaccine since 1995, possibly because the vaccine contains live vaccinia, which is associated with potential adverse events [4].

In 1999, Jackman and colleagues reported the successful production of a recombinant gp350 vaccine in Chinese hamster ovary cells and showed that it elicited gp350 and neutralizing antibodies in rabbits [5]. An EBV vaccine containing this antigen was subsequently employed in four clinical trials. A phase 1 study evaluated the safety and immunogenicity of a 3-dose regimen of vaccine containing 50 µg of gp350 given intramuscularly [6]. EBV

antibody-negative and antibody-positive subjects 18 to 25 years of age were randomized to receive the vaccine adjuvanted with 3-O-desacyl-4'-monophosphoryl lipid A and aluminum salt known as Adjuvant System 04 (AS04) or aluminum salt alone. A phase 1/2 study randomized EBV-naïve subjects 18 to 37 years old to receive unadjuvanted vaccine, vaccine adjuvanted with AS04, or vaccine adjuvanted with aluminum salt only. The aggregate data from 138 subjects showed that the vaccine was safe with one notable exception. Ten days after receiving a second dose of vaccine adjuvanted with AS04, an EBV antibody-positive subject was hospitalized for an apparent autoimmune reaction consisting of meningismus and arthritis of the knees, ankles and lower back. The immunogenicity data, which included measurement of gp350 and neutralizing antibodies, indicated that vaccine adjuvanted with AS04 was superior to non-adjuvanted vaccine and better than vaccine adjuvanted with aluminum salt.

The third trial was a phase 2, placebo-controlled, double-blind study evaluating safety, immunogenicity, and efficacy of recombinant gp350 vaccine in EBV-naïve young adults ages 16 to 25 [7•]. The vaccine contained 50 µg of gp350 and 50 µg of AS04 in a 0.5 mL volume that was given intramuscularly at 0, 1 and 5 months. There were no significant adverse events and 76/77 (98.7%) of vaccinees who were not subsequently infected by wild-type EBV developed gp350 antibodies. The efficacy analysis consisted of following the subjects for up to 19 months postimmunization for evidence of EBV infection and infectious mononucleosis. The vaccine did not prevent infection: 13 (14%) of 90 vaccine recipients became infected versus 18 (20%) of 91 placebo subjects. However, it had a significant effect on clinical disease. In the intent-to-treat population, infectious mononucleosis developed in 2 (2%) of 90 vaccinees as compared with 9 (10%) of 91 placebo recipients ($P=0.03$, Fisher exact test, 1-sided). The importance of this will be emphasized later when the prospect that an EBV vaccine could prevent Hodgkin lymphoma or MS is discussed. Unfortunately, no further trials of this vaccine have been reported.

Finally, a phase 1 study of recombinant gp350 vaccine with an aluminum hydroxide adjuvant was conducted in 16 pediatric renal transplant candidates [8]. Subcutaneous dosing regimens of 12.5 µg or 25 µg of gp350 given 3 or 4 times over a total of 32 weeks were well tolerated. All 13 evaluable subjects mounted an anti-gp350 antibody response but only four made a neutralizing antibody response. Because there was no control group, vaccine efficacy could not be assessed but this small phase 1 trial did show that immunization of children awaiting transplantation for chronic renal disease is feasible.

A different vaccine strategy is to control expansion of EBV-infected B cells by generating CD8⁺ T-cell immunity to EBNA3A [9]. The potential role of these viral proteins in B cell transformation precludes their use in whole protein based vaccines, and therefore a peptide vaccine was generated and tested in adult volunteers. This trial utilized an EBNA3A peptide epitope (FLRGRAYGL) restricted by HLA B8 [10] with tetanus toxoid formulated in a water-in-oil adjuvant as a source of T-cell help [11•]. EBV-naïve individuals were immunized on a two-month interval schedule. Of the 14 enrolled subjects, four received placebo, two were immunized with 50 µg dose of peptide and the remaining 8 individuals were immunized with a 5 µg dose of peptide. This strategy was effective at generating a peptide-specific CD8⁺ T-cell response in most individuals as measured by *ex vivo* peptide

specific interferon gamma production. Among subjects who subsequently acquired wild-type EBV infection, infectious mononucleosis occurred in 1 of 2 subjects in the placebo group versus 0 of 4 in the vaccinated cohort, hinting that this vaccine might prevent symptomatic EBV infection.

The general utility of epitope vaccines is limited by the fact that they only target specific HLA types. Nonetheless epitope vaccines might be useful for patients with PTLN, where the HLA type is known. This trial was also a “proof-of-principle,” which showed that EBV vaccines that generate CD8⁺ T-cell immunity were safe and did not predispose subjects to disease after primary infection by wild type EBV infection.

Progress: Therapeutic EBV vaccines

Therapeutic vaccines to treat EBV-associated malignancies are also in development. The goal is to enhance recognition of tumor cells through their expression of viral antigens. A recent phase 1 dose-escalation study was conducted in 18 subjects with nasopharyngeal carcinoma [12••]. The vaccine was a modified vaccinia Ankara recombinant vector expressing the tumor-associated viral antigens EBNA-1 and LMP-2. Subjects received one of 5 doses ranging from 5×10^7 to 5×10^8 pfu intradermally at 3-week intervals for a total of three inoculations. The vaccine was well tolerated with no evidence of dose-limiting toxicity. Overall, 15 of 18 patients developed T-cell responses to one or both vaccine antigens. There was a dose-response relationship with the highest dose eliciting the strongest response and thus Phase 2 studies are planned using the highest dosage regimen.

Prospects: What EBV vaccines could achieve

The global burden of EBV-associated diseases is enormous. EBV vaccines could reduce this burden significantly. How can they best be evaluated going forward? For prophylactic vaccines, ideal targets for efficacy trials in order of probable success are infectious mononucleosis, PTLN, and endemic Burkitt lymphoma [13]. A vaccine containing gp350 antigen is a logical candidate because EBV causes infection predominantly by binding its major envelope glycoprotein gp350 to the CD21 receptor on the surface of B lymphocytes [14, 15]. A neutralizing antibody response, which can be measured, for example, by inhibition of EBV transformation of B lymphocytes [5, 15], is closely associated with the gp350-specific antibody response [16]. Neutralizing antibody presumably is our best humoral defense against cell entry by EBV.

The recommendation to use a gp350-based vaccine is more than just theoretical. Infection of rhesus macaques with rhesus lymphocryptovirus reproduces many of the features of human EBV infection including long periods of viral shedding, viral latency, and the propensity to develop EBV associated malignancies if immunosuppressed [17]. A recent study showed that a soluble gp350 vaccine protected the animals from infection after oral challenge and reduced blood levels of viral DNA for up to two years after infection with the challenge virus [18•].

In addition, gp350 vaccine has been shown to protect against infectious mononucleosis in a phase 2 clinical trial [7••]. Targeting infectious mononucleosis for the first phase 3 efficacy

trial makes sense because a susceptible population with a high incidence of disease, namely college freshmen, is at hand. In a prospective study of EBV-naïve University of Minnesota undergraduates, the incidence of primary EBV infection among freshmen was 26% and the incidence of infectious mononucleosis was 22% [19••]. A vaccine trial to prevent infectious mononucleosis should include viral load measurements on both vaccinees and placebo subjects who contract wild type EBV after vaccination, because there is a statistically significant correlation between the quantity of virus in the blood and disease severity [19, 20]. If the vaccine is shown to reduce blood viral load as well as clinical symptoms, the likelihood that it could prevent malignancies becomes stronger. Increased blood viral loads have been associated with the development of PTLT [21], and increased plasma EBV DNA is concordant with outcome in Hodgkin lymphoma [22]. To gain insight into the duration of protection, it would also be worthwhile to evaluate EBV gp350-specific memory T-cell responses in vaccinees and to determine whether T-cells in vaccinated individuals undergo EBV-induced changes in IL-15 receptor expression.

PTLT is the next logical disease to study because EBV-naïve hematopoietic stem cell or solid organ transplant candidates who are at the highest risk could be identified and vaccinated pretransplant. Such a study should also analyze the effect of vaccination on the quantity and duration of EBV DNAemia, which could be responsible for posttransplant morbidity in the absence of full-blown PTLT. Endemic Burkitt lymphoma and nasopharyngeal carcinoma are serious malignancies and hence worthy targets for preventive trials. Burkitt lymphoma is the better candidate for a vaccine field trial because its “incubation period” is shorter and there are distinct high-incidence geographic foci where investigations could be carried out [23]. EBV vaccine might also reduce the incidence of Hodgkin lymphoma. The gp350 vaccine has been shown to protect against infectious mononucleosis and a history of infectious mononucleosis increases the risk of EBV-related Hodgkin lymphoma by more than 3-fold [24].

An EBV vaccine could conceivably reduce the incidence and/or severity of MS, which is arguably the most common neuroinflammatory disease worldwide. There is strong evidence that EBV is an important environmental risk factor for MS. Nearly 100% of MS patients have EBV antibody [25]; their antibody levels are markedly elevated especially against EBNA-1 [26]; a history of infectious mononucleosis increases the risk of developing MS [27]; EBV-specific CD8+ T cell responses are elevated during active MS [28]; and, finally, therapy with monoclonal antibodies that deplete the B cell reservoir of latent EBV virus has a beneficial effect in MS [29].

Therapeutic vaccines to treat EBV-associated cancers are in their early stages. The trial by Hui and colleagues showing that an EBV vaccine elicited T-cell responses to the latent antigens EBNA-1 and/or LMP-2 in 15/18 subjects with nasopharyngeal carcinoma is encouraging and has prompted a phase 2 protocol [12].

Problems in EBV vaccine development

EBV vaccines have many promising prospects, but these cannot be fulfilled until some problems and knowledge gaps are overcome. The major problem in EBV vaccine

development is to find an industry sponsor willing to invest in it. A sponsor, if found, faces challenges to implementing successful field trials in humans, which cannot be preceded by animal studies because a practical animal model does not exist. The optimum vaccine formulation, including both the antigen construct and adjuvant, needs to be determined. Would a gp350 vaccine with additional EBV antigens be more effective than gp350 alone [30]? Could better adjuvants be incorporated? If the first phase 3 trial is to prevent infectious mononucleosis, in which case the major beneficiaries would be EBV antibody-negative individuals, what is the ideal age group to test? Age-specific EBV antibody prevalence data from the National Health and Examinations Surveys indicate that white children could be vaccinated as late as 12 years of age, whereas non-whites need to be vaccinated before they are 6 years old [31].

A gp350-based vaccine that raises neutralizing antibodies may not be appropriate for all suggested indications and more than one vaccine may need to be developed. For example, vaccines that induce T-cell responses to antigens in tumor cells, especially EBNA and LMPs, may be required to prevent or treat EBV-associated malignancies.

Conclusion

EBV is a common worldwide human pathogen that causes acute and chronic infections and cancers. It is intimately associated with immune-mediated diseases especially MS. EBV vaccine could prevent or reduce the severity of some, if not all, of these conditions. Nevertheless, an EBV vaccine is not yet available for general use. The challenge is to assemble a group of individuals dedicated to its development and identify the resources needed so that EBV vaccine can be given to all those who could benefit from it.

Acknowledgments

This work was supported by grants from the University of Minnesota International Center for Antiviral Research and Epidemiology, the University of Minnesota Foundation, and the National Institutes of Health (2PO1 DK 13083).

Abbreviations

AS04	adjuvant system 04
EBV	Epstein-Barr virus
EBNAs	Epstein-Barr virus nuclear antigens
gp	glycoprotein
HLA	human leukocyte antigen
LMP	latent membrane protein
MS	multiple sclerosis
PTLD	posttransplant lymphoproliferative disorder

References and recommended reading

Papers of particular interest have been highlighted as:

•• of special interest

•• of outstanding interest

1. Epstein MA. Epstein-Barr virus--is it time to develop a vaccine program? *J Natl Cancer Inst.* 1976; 56:697–700. [PubMed: 176406]
2. Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. *Sci Transl Med.* 2011; 3:107fs7.
3. Gu SY, Huang TM, Ruan L, Miao YH, Lu H, Chu CM, Motz M, Wolf H. First EBV vaccine trial in humans using recombinant vaccinia virus expressing the major membrane antigen. *Dev Biol Stand.* 1995; 84:171–177. [PubMed: 7796951]
4. Maurer DM, Harrington B, Lane JM. Smallpox vaccine: contraindications, administration, and adverse reactions. *Am Fam Physician.* 2003; 68:889–896. [PubMed: 13678138]
5. Jackman WT, Mann KA, Hoffmann HJ, Spaete RR. Expression of Epstein-Barr virus gp350 as a single chain glycoprotein for an EBV subunit vaccine. *Vaccine.* 1999; 17:660–668. [PubMed: 10067671]
6. Moutschen M, Leonard P, Sokal EM, Smets F, Haumont M, Mazzu P, Bollen A, Denamur F, Peeters P, Dubin G, et al. Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein-Barr virus vaccine in healthy adults. *Vaccine.* 2007; 25:4697–4705. [PubMed: 17485150]
- 7••. Sokal EM, Hoppenbrouwers K, Vandermeulen C, Moutschen M, Leonard P, Moreels A, Haumont M, Bollen A, Smets F, Denis M. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein-Barr virus vaccine in healthy young adults. *J Infect Dis.* 2007; 196:1749–1753. The first phase 2 trial of gp350 EBV vaccine that demonstrated safety, immunogenicity and protection against infectious mononucleosis. [PubMed: 18190254]
8. Rees L, Tizard EJ, Morgan AJ, Cubitt WD, Finerty S, Oyewole-Eletu TA, Owen K, Royed C, Stevens SJ, Shroff RC, et al. A phase I trial of Epstein-Barr virus gp350 vaccine for children with chronic kidney disease awaiting transplantation. *Transplantation.* 2009; 88:1025–1029. [PubMed: 19855249]
9. Khanna R, Burrows SR, Kurilla MG, Jacob CA, Misko IS, Sculley TB, Kieff E, Moss DJ. Localization of Epstein-Barr virus cytotoxic T cell epitopes using recombinant vaccinia: implications for vaccine development. *J Exp Med.* 1992; 176:169–176. [PubMed: 1377222]
10. Burrows SR, Sculley TB, Misko IS, Schmidt C, Moss DJ. An Epstein-Barr virus- specific cytotoxic T cell epitope in EBV nuclear antigen 3 (EBNA 3). *J Exp Med.* 1990; 171:345–349. [PubMed: 1688611]
- 11•. Elliott SL, Suhrbier A, Miles JJ, Lawrence G, Pye SJ, Le TT, Rosenstengel A, Nguyen T, Allworth A, Burrows SR, et al. Phase I trial of a CD8+ T-cell peptide epitope-based vaccine for infectious mononucleosis. *J Virol.* 2008; 82:1448–1457. A feasibility trial that demonstrated safety and immunogenicity of an EBV CD8+ T-cell peptide vaccine. While not practical for general use because of HLA restriction, it could be studied in subjects whose HLA is well characterized, such as pretransplant candidates. [PubMed: 18032491]
- 12••. Hui EP, Taylor GS, Jia H, Ma BB, Chan SL, Ho R, Wong WL, Wilson S, Johnson BF, Edwards C, et al. Phase I trial of recombinant modified vaccinia Ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. *Cancer Res.* 2013; 73:1676–1688. A thoroughly designed trial whose hypothesis is that enhanced recognition of tumor cells through their expression of EBNA antigens EBNA-1 and LMP-2 will be therapeutic. Safety, immunogenicity, and a dose-response effect were demonstrated. [PubMed: 23348421]
13. Balfour HH Jr. Epstein-Barr virus vaccine for the prevention of infectious mononucleosis--and what else? *J Infect Dis.* 2007; 196:1724–1726. [PubMed: 18190249]

14. Nemerow GR, Mold C, Schwend VK, Tollefson V, Cooper NR. Identification of gp350 as the viral glycoprotein mediating attachment of Epstein-Barr virus (EBV) to the EBV/C3d receptor of B cells: sequence homology of gp350 and C3 complement fragment C3d. *J Virol.* 1987; 61:1416–1420. [PubMed: 3033269]
15. Moss DJ, Pope JH. Assay of the infectivity of Epstein-Barr virus by transformation of human leucocytes in vitro. *J Gen Virol.* 1972; 17:233–236. [PubMed: 4344316]
16. Sashihara J, Burbelo PD, Savoldo B, Pierson TC, Cohen JI. Human antibody titers to Epstein-Barr Virus (EBV) gp350 correlate with neutralization of infectivity better than antibody titers to EBV gp42 using a rapid flow cytometry-based EBV neutralization assay. *Virology.* 2009; 391:249–256. [PubMed: 19584018]
17. Moghaddam A, Rosenzweig M, Lee-Parritz D, Annis B, Johnson RP, Wang F. An animal model for acute and persistent Epstein-Barr virus infection. *Science.* 1997; 276:2030–2033. [PubMed: 9197263]
18. Sashihara J, Hoshino Y, Bowman JJ, Krogmann T, Burbelo PD, Coffield VM, Kamrud K, Cohen JI. Soluble rhesus lymphocryptovirus gp350 protects against infection and reduces viral loads in animals that become infected with virus after challenge. *PLoS Pathog.* 2011; 7:e1002308. An important study in which rhesus macaques were protected from an EBV challenge infection after receiving gp350 vaccine. [PubMed: 22028652]
19. Balfour HH Jr, Odumade OA, Schmeling DO, Mullan BD, Ed JA, Knight JA, Vezina HE, Thomas W, Hogquist KA. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. *J Infect Dis.* 2013; 207:80–88. A prospective study demonstrating the significant disease burden of primary EBV infection in EBV-naïve college freshmen. Such students are ideal subjects for a vaccine study whose primary endpoint is protection from infectious mononucleosis. [PubMed: 23100562]
20. Balfour HH Jr, Holman CJ, Hokanson KM, Lelonek MM, Giesbrecht JE, White DR, Schmeling DO, Webb C, Cavert W, Wang DH, et al. A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. *J Infect Dis.* 2005; 192:1505–1512. [PubMed: 16206064]
21. Holman CJ, Karger AB, Mullan BD, Brundage RC, Balfour HH Jr. Quantitative Epstein-Barr virus shedding and its correlation with the risk of post-transplant lymphoproliferative disorder. *Clin Transplant.* 2012; 26:741–747. [PubMed: 22385033]
22. Kanakry JA, Li H, Gellert LL, Lemas MV, Hsieh WS, Hong F, Tan KL, Gascoyne RD, Gordon LI, Fisher RI, et al. Plasma Epstein-Barr virus DNA predicts outcome in advanced Hodgkin lymphoma: correlative analysis from a large North American cooperative group trial. *Blood.* 2013; 121:3547–3553. [PubMed: 23386127]
23. Rainey JJ, Omenah D, Sumba PO, Moormann AM, Rochford R, Wilson ML. Spatial clustering of endemic Burkitt's lymphoma in high-risk regions of Kenya. *Int J Cancer.* 2007; 120:121–127. [PubMed: 17019706]
24. Hjalgrim H, Rostgaard K, Johnson PC, Lake A, Shield L, Little AM, Ekstrom-Smedby K, Adami HO, Glimelius B, Hamilton-Dutoit S, et al. HLA-A alleles and infectious mononucleosis suggest a critical role for cytotoxic T-cell response in EBV-related Hodgkin lymphoma. *Proc Natl Acad Sci U S A.* 2010; 107:6400–6405. [PubMed: 20308568]
25. Sumaya CV, Myers LW, Ellison GW. Epstein-Barr virus antibodies in multiple sclerosis. *Arch Neurol.* 1980; 37:94–96. [PubMed: 6243930]
26. Munger KL, Levin LI, O'Reilly EJ, Falk KI, Ascherio A. Anti-Epstein-Barr virus antibodies as serological markers of multiple sclerosis: a prospective study among United States military personnel. *Mult Scler.* 2011; 17:1185–1193. [PubMed: 21685232]
27. Handel AE, Williamson AJ, Disanto G, Handunnetthi L, Giovannoni G, Ramagopalan SV. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *PLoS One.* 2010; 5.
28. Angelini DF, Serafini B, Piras E, Severa M, Coccia EM, Rosicarelli B, Ruggieri S, Gasperini C, Buttari F, Centonze D, et al. Increased CD8+ T cell response to Epstein-Barr virus lytic antigens in the active phase of multiple sclerosis. *PLoS Pathog.* 2013; 9:e1003220. [PubMed: 23592979]

29. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med*. 2008; 358:676–688. [PubMed: 18272891]
30. Cohen JI, Mocarski ES, Raab-Traub N, Corey L, Nabel GJ. The need and challenges for development of an Epstein-Barr virus vaccine. *Vaccine*. 2013; 31 (Suppl 2):B194–196. [PubMed: 23598481]
31. Balfour HH Jr, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W. Age- specific prevalence of Epstein-Barr virus infection among individuals aged 6–19 years in the United States and factors affecting its acquisition. *J Infect Dis*. 2013; 208:1286–1293. [PubMed: 23868878]

Highlights

- Prophylactic and therapeutic EBV vaccines have been tested but none is licensed.
- Best progress to date: prevention of infectious mononucleosis by a gp350 vaccine.
- Problems are lack of an animal model and finding the best immunogen and adjuvant.
- Prospects include prevention of mono, PTLN, MS, and treatment of EBV-related cancer.

Table 1

Prospects, progress, and problems in EBV vaccine development

Prospects	Progress	Problems
Prevention of infectious mononucleosis	Infectious mononucleosis was prevented in a phase 2 study with a subunit gp350 vaccine [7]. A CD8+ T-cell peptide vaccine was immunogenic with a hint of efficacy [11].	gp350: Duration of protection unknown. Viral loads and T-cell specific responses were not evaluated. The ideal age at which to vaccinate may differ according race/ethnicity and socioeconomic. CD8+ T-cell peptide vaccine: HLA restricted.
Prevention of nasopharyngeal carcinoma	A vaccinia construct expressing EBV membrane glycoprotein was immunogenic and may have reduced incidence of EBV infection in Chinese children [3].	Long incubation period from EBV infection to development of nasopharyngeal carcinoma makes efficacy trials impractical.
Prevention of lymphomas	A subunit gp350 vaccine was safe in pediatric renal transplant candidates [8].	Vaccine was poorly immunogenic probably due to low dose and weak adjuvant; trial could not assess protection from PTLD.
Treatment of nasopharyngeal carcinoma	A vaccinia recombinant vector expressing the tumor-associated viral antigens EBNA-1 and LMP-2 was safe and immunogenic [12].	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 [28]; monoclonal antibodies that deplete the B cell reservoir of latent EBV virus were beneficial in MS [29].	Long incubation period from EBV infection to MS makes vaccine efficacy trials impractical except perhaps in first-degree relatives.