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REVIEW ARTICLE

The promise of a prophylactic Epstein–Barr virus vaccine

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The worldwide burden of disease due to Epstein–Barr virus (EBV) infection is enormous. Diseases include endemic Burkitt lymphoma, infectious mononucleosis, cancers after transplantation, Hodgkin lymphoma, and nasopharyngeal carcinoma. A prophylactic EBV vaccine has the potential to significantly reduce the incidence and/or the severity of all these diseases. Infectious mononucleosis can be nasty and prolonged with a median duration of 17 days. Patients, especially children, undergoing bone marrow or solid organ transplantation may develop post-transplant lymphoproliferative disorder (PTLD). Preventing or modifying primary EBV infection could reduce the incidence PTLD, and also certain lymphomas and nasopharyngeal carcinoma. EBV is a major environmental risk factor for multiple sclerosis (MS). Contracting EBV is essential to getting MS, and having a childhood case of infectious mononucleosis increases that risk. Vaccinating against EBV could be vaccinating against MS.

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INTRODUCTION

Epstein–Barr virus (EBV), also known as human herpesvirus 4, was discovered in lymphoma cells in 1964 by Epstein et al.,[1] thus making it the first recognized human cancer virus. Nine years later, in 1973, Epstein and Achong[2] proposed a rationale for developing a prophylactic EBV vaccine. Yet, more than four decades later, there are no licensed EBV vaccines even though the worldwide burden of EBV disease is immense. This review describes the potential benefits of a prophylactic EBV vaccine, and discusses the odyssey of its development.

DISEASE TARGETS FOR A PROPHYLACTIC EBV VACCINE

EBV has been associated with a farrago of inflammatory and malignant diseases. Those most likely, in our opinion, to be prevented or modified by a prophylactic EBV vaccine are described in this section.

Endemic Burkitt lymphoma

EBV was discovered by examining Burkitt lymphoma cells under an electron microscope (Fig. 1). This landmark event is quite fascinating as told by Tony Epstein. The direct quotes that follow are from Epstein's chapter in E.S. Robertson's book, "Epstein–Barr Virus".[3] As the story goes, Denis Burkitt, a British surgeon, was stationed in East Africa during World War II, and requested to remain there after the war. In 1957, while working in Mulago Hospital, Kampala, Uganda, he was consulted about a child with unusual swellings in all four angles of the jaw. Shortly thereafter, he saw another child with an identical condition, and this prompted him to search through the hospital's medical records for similar cases. The records revealed that tumors of the jaw were quite common in young children in Uganda. Burkitt published a paper in 1958 documenting 38 cases of the disease,[4] later known as Burkitt lymphoma, but this article went largely unnoticed.

A serendipitous incident brought Burkitt and Epstein together. Epstein was studying Rous sarcoma virus at the Middlesex Hospital in London. Burkitt had connections with surgeons at Middlesex Hospital and when he was home on leave they customarily invited him to lecture about his experiences in Uganda. In 1961, Burkitt lectured on "The commonest children's cancer in Tropical Africa: a hitherto unrecognized syndrome." Epstein saw the title of the talk on a notice board at the hospital and, in his words, "for reasons to this day I am unsure about, but probably curiosity, I attended." The details in the talk caused Epstein, with his background in tumor viruses, to postulate that this condition might be caused by a cancer virus. In a meeting several days later, Burkitt agreed to send biopsy samples from his patients for Epstein to work on in London.

For almost 2 years, the standard techniques of viral isolation available at that time were tried on the lymphoma samples and failed. Then, a very fortunate incident turned failure into success. On 6 December 1963, the plane from Kampala carrying a biopsy sample was diverted from London to Manchester because of fog and the biopsy could not be retrieved until the plane was able to land at London Airport in late afternoon. Alas! the fluid in which the biopsy was suspended was cloudy and the natural assumption was that the specimen had been contaminated by bacteria due to the prolonged journey. However, instead of discarding the material, Epstein examined it directly as a wet preparation under a light microscope. Voila! The cloudiness was due to "a large number of round, viable looking free-floating tumor cells which must have been shaken free during transit from the cut edges of the lymphoma sample." In other words, a suspension culture of tumor cells had started itself and was subsequently propagated as a continuous line of Burkitt lymphoma cells.

As soon as some cells could be spared from the suspension culture, they were prepared for electron microscopy and examined by Epstein on 24 February 1964. Epstein was

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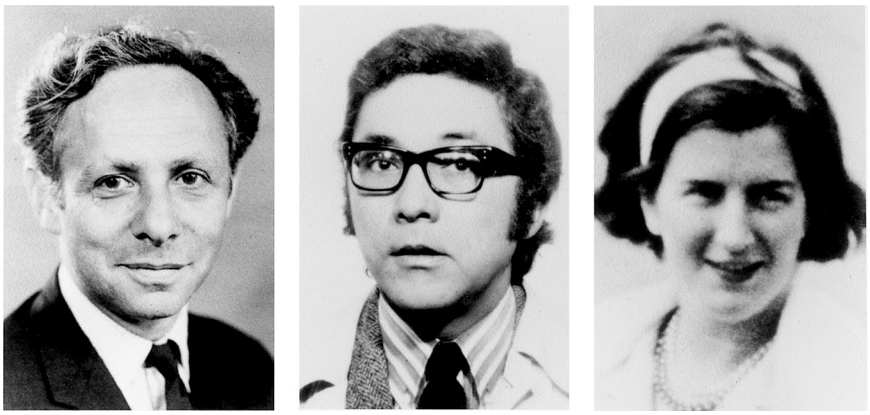

Fig. 1 Sir Anthony Epstein, Bert Achong, and Yvonne Barr (photo courtesy of Sir Anthony Epstein)

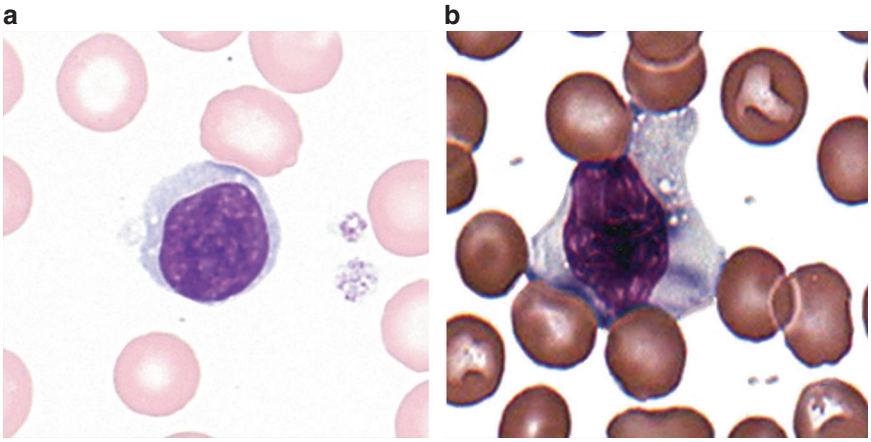

Fig. 2 **a** Normal peripheral blood lymphocyte: clompy chromatin, high nucleus-to-cytoplasm ratio, scant blue cytoplasm. **b** Atypical/reactive peripheral blood lymphocyte: Large cell with high cytoplasm-to-nucleus ratio, basophilic cytoplasm showing radial basophilia, azure granules, vacuoles, and slightly indented nucleus. Images courtesy of S.M. Wiesner

"exhilarated to observe unequivocal viral particles in a cultured [Burkitt lymphoma] cell in the very first grid square to be searched…I recognized the virus at once as having the typical morphology of the herpes group." Thus, EBV became the first recognized human cancer virus.

Endemic Burkitt lymphoma is not just of historical interest, but it remains a major cause of childhood cancer in East Africa, where it is endemic. A recent study from Malawi reported that between 2011 and 2013, 74 (65%) of 114 lymphomas in children 2–16 years of age were Burkitt lymphoma.[5]

Endemic Burkitt lymphoma is a good target for preventive vaccine trials because of its gravity, its relatively short incubation period (as compared with nasopharyngeal carcinoma (NPC) for example), and the existence of distinct geographic foci with a high incidence of Burkitt lymphoma.[6]

Infectious mononucleosis

Infectious mononucleosis was first recognized as a clinical entity in the 1880s by Nil Filatov, who is considered to be the founder of Russian pediatrics. He called the illness "idiopathic adenitis."[7] A German pediatrician, Emil Pfeiffer, described this condition at about the same time,[8] and called it "Drüsenfieber" (glandular fever). Pfeiffer recognized that the illness mainly involved the lymph glands, especially the cervical lymph nodes, and that recovery was the rule. Sprunt and Evans[9] put the clinical and hematologic findings together in 1920. They described six young adults, all in their 20s, "presenting the symptoms of an acute infection, a moderate enlargement of the lymph nodes and of the spleen, and a mononuclear lymphocytosis," and coined the name, infectious mononucleosis.

Distinguishing infectious mononucleosis from acute lymphocytic leukemia was a dilemma. To help solve this, a student health physician (C.A. McKinlay) and a clinical pathologist (Hal Downey) reported nine university students with acute infectious mononucleosis and carefully detailed the morphology of their circulating lymphocytes.[10] The characteristic features of these cells, often referred to as Downey cells or atypical lymphocytes, are their large size, clear cytoplasm, and a folded or indented nucleus (Fig. 2). We now know that these are $CD8^+$ T lymphocytes reacting against EBV-infected B cells.[11]

A blood test to diagnose infectious mononucleosis was discovered by Paul and Bunnell in 1932.[12] They found "rather high concentrations" of antibodies against sheep red blood cells in 4 patients with infectious mononucleosis, whereas such concentrations were rarely present among 275 patients with a variety of other diseases. Paul and Bunnell defined these as heterophile antibodies, which have "the capacity to react with certain antigens, which are quite different from, and phylogenetically unrelated to, the one instrumental in producing the antibody response." Most of today's point-of-care tests for infectious mononucleosis detect heterophile antibodies against a variety of mammalian red blood cells.

The connection between EBV and infectious mononucleosis was made in the following way. In the mid-1960s, Werner and Gertrude Henle had acquired Burkitt lymphoma cells from the Epstein laboratory and were trying to establish lymphocyte cell

lines from them without success. A technologist working in their laboratory regularly donated lymphocytes for EBV transmission/transformation experiments, but her cells did not survive in culture.[13] Serendipitously, she became ill in August 1967 and developed a rubella-like rash. Her physician's differential diagnosis was rubella versus infectious mononucleosis. Her rubella antibodies were negative, but her heterophile antibody test was positive. Her diagnosis was therefore infectious mononucleosis. It turns out that she had been treated with ampicillin, which is known to cause a rash as a reflection of transient penicillin hypersensitivity during the acute stage of infectious mononucleosis.[14] After she returned to work, her lymphocytes now grew continuously in culture and were positive for EBV antigens. She also had acquired EBV-specific antibodies, which was strong evidence that EBV caused infectious mononucleosis. To conclusively prove the point, additional serum samples were obtained from biobanks that contained pre- and post-illness samples. Samples from college students were especially valuable to prove conclusively that primary EBV infection caused infectious mononucleosis.[15]

Infectious mononucleosis is an excellent target for initial trials of a prophylactic vaccine because the incidence is high in young adults. Our prospective studies have shown that 25% of EBV-naive college students are infected during their freshman year and 20% of them develop infectious mononucleosis.[16,17] The illness is relatively long (median duration, 17 days) and can be debilitating. The acceptability of a preventive EBV vaccine is high among university students. A recent cross-sectional study found that 72% of University of Minnesota freshmen (161/223) believed they would benefit from such a vaccine.[18]

Post-transplant lymphoproliferative disorder

In the 1970s, the prevalence of EBV in the oropharynx was recognized to be much higher among patients with malignancies or solid organ allografts than in the general population, implicating EBV in the pathogenesis of lymphoma and post-transplant lymphoproliferative disorder (PTLD).[19,20] In 1981, Hanto et al.[21] presented virologic data convincingly showing EBV to be the cause of at least some cases of PTLD.

Management of PTLD was challenging then and it still is today. Before acyclovir was Food and Drug Administration approved, we were fortunate to obtain it to treat a 12-year-old boy who developed PTLD after a kidney transplant.[22] He responded well to intravenous acyclovir therapy while his lymphoproliferation was polyclonal, but when it eventually became monoclonal he became refractory to therapy and died.

EBV-naive recipients of either solid organ or hematopoietic cell transplants, most often children, are at risk for PTLD and could potentially benefit from a prophylactic vaccine. Because the incidence of PTLD is low, a vaccine trial more than likely would need to be conducted at multiple sites.

Hodgkin lymphoma

EBV has been associated with ~40% of Hodgkin lymphomas as documented by finding EBV RNA or EBV protein in lymphoma cells using in situ hybridization or immunohistochemistry techniques.[23] Curiously, a link between infectious mononucleosis and Hodgkin lymphoma was suspected, based on clinical, hematological, and serological characteristics, years before EBV was discovered to be a cause of both of them.[24,25]

Infectious mononucleosis has clearly been established as a risk factor for Hodgkin lymphoma with a median time from onset of infectious mononucleosis to lymphoma of 2.9 years.[26,27] What is not clear, as succinctly stated by Ambinder, is "whether primary infection per se is the risk factor…or whether primary symptomatic infection is the risk factor.[28] This is a huge consideration for vaccine design. A vaccine that does not prevent infection but reduces or eliminates symptoms would be ideal if the risk is symptomatic infection. On the other hand, a sterilizing vaccine would be best if primary infection without symptoms is also a risk. A vaccine trial whose endpoint is prevention of Hodgkin lymphoma is impractical because of the large number of participants required, and the necessity for an inordinately long follow-up period. However, if EBV vaccine were to become universally used in pediatrics, the potential is there for a substantial reduction of cases of Hodgkin lymphoma.

Nasopharyngeal carcinoma

There are several WHO classifications for NPC. Non-keratinizing, undifferentiated squamous cell carcinoma is the most common subtype in adults and children, and also the one most highly associated with EBV.[29,30] NPC has a unique geographical distribution.[31] Areas of high incidence include China, especially Southern China, the Arctic, and Northern Africa.[30,31] Other risk factors are race, family, male sex, and possibly diet.[30]

The association of NPC with EBV was first appreciated by Old et al.,[32] who tested 352 serum samples and found that 64/94 patients (68%) with either NPC or Burkitt lymphoma had precipitin antibodies against an antigen derived from Burkitt lymphoma cells, whereas these antibodies were found in only 30/258 persons (12%) who were healthy or had other diseases ($P < 0.0001$, Fisher's exact test, two sided). zur Hausen et al.[33] expanded on these results by demonstrating the presence of EBV DNA in biopsies from Burkitt lymphoma and NPC.

The EBV antibody profile of NPC patients is characterized by relatively high levels of circulating IgA,[34] which have been shown to be EBV specific and to increase as disease progresses.[35] EBV DNA levels in the plasma or serum can also be used to monitor disease progression.[36]

NPC has been a target for therapeutic EBV vaccines, which have shown modest success.[37,38] Because of the lengthy period from primary EBV infection to NPC, a vaccine trial to prevent NPC is impractical. That having been said, vaccination of children in high-risk geographical regions could have an enormous public health benefit if it protects vaccinees and provides herd immunity against NPC.

Multiple sclerosis

Five lines of evidence support the concept that EBV is the major environmental risk factor for developing multiple sclerosis (MS). Essentially all MS patients have been infected by EBV,[39] their EBV-specific antibody levels are elevated, especially against EBV nuclear antigen-1 (EBNA-1),[40,41] a history of infectious mononucleosis increases the risk of developing MS,[42] EBV-specific $CD8^+$ T cell responses are elevated in active MS,[43] and EBV antigens indicative of viral replication are present in the brain tissue of MS patients.[44,45]

Furthermore, EBV-specific adoptive T cell therapy has shown promise in modifying the severity of MS, which supports the notion that active EBV infection is responsible, at least in part, for disease progression.[46,47]

Because the period from primary EBV infection to MS is usually several decades, a vaccine trial to prevent MS is impractical. However, if EBV vaccine is shown to be effective in other field trials, immunizing relatives of MS patients should be a high priority. For example, a study in Denmark, a high-incidence area of MS, found that first-degree relatives of MS patients had a sevenfold increased risk of MS as compared with the background population.[48]

Chronic active EBV infection

EBV typically infects B cells or epithelial cells. Infection of T lymphocytes or natural killer (NK) cells is uncommon, but when it occurs, serious diseases ensue. The WHO includes chronic active EBV infection (CAEBV) under the classification of EBV^+

T cell and NK cell lymphomas.[49] CAEBV has been reported most frequently in Japanese children, but it does occur in the United States and can present in adults as well as children.[50]

Arai[51] describes CAEBV as "characterized by clonal proliferation of EBV-infected T or NK cells and their infiltration into systemic organs, leading to their failure. Inflammatory symptoms, fever, lymphadenopathy, and liver dysfunction are main clinical findings". Patients have persistently elevated levels of EBV DNA in the blood, and T cells or NK cells can be shown to be infected by EBV.[51]

Antivirals, immune modulators, and cytotoxic drugs have not been effective treatment for CAEBV. While hematopoietic cell transplantation or immunotherapy holds promise, they are not practical treatments on a large scale due to cost and complications of the therapies. It is possible that EBV vaccine could prevent CAEBV, especially for patients who present with infectious mononucleosis, which has been shown to occur less frequently in EBV vaccinees as compared with placebo recipients.[52]

Chronic infectious mononucleosis

We have observed two patterns of chronic infectious mononucleosis. The first and more common is recovery from the initial disease, but recurrence of symptoms months to years later. The second pattern is a continuous "mono-like" illness that lasts indefinitely. Both patterns occur in children as well as adults and are more common in females. Symptoms include, in order of frequency: fatigue, weakness, joint pain, susceptibility to infections, diminished cognition, thyroid disorders, hypersomnia, and tender cervical lymphadenopathy. The usual virology laboratory profile is: negative for EBV DNA in blood; very high IgG plasma or serum antibody levels against EBV viral capsid antigen (VCA); modestly elevated plasma or serum antibody levels against EBV nuclear antigen-1 (EBNA-1); and the absence of circulating VCA IgM antibodies.

There is no evidence-based treatment, but we have reported modest success with a combination of antiviral drugs and an anti-inflammatory diet as posted on our website (http://z.umn.edu/ebvdiseases).[53] Because nearly all of these patients report having infectious mononucleosis, a vaccine that prevents the primary illness logically could also prevent chronic infectious mononucleosis.

DEVELOPMENT OF A PROPHYLACTIC EBV VACCINE: WHERE ARE WE NOW?

EBV vaccine holds the promise of preventing or modifying the severity of all the diseases listed in Table 1. Yet, more than 4 decades after development of a prophylactic EBV vaccine was advocated,[2,54] a licensed vaccine does not exist. Why?

The reasons are not entirely clear, but likely include: skepticism about what an EBV vaccine could actually achieve; the impression that infectious mononucleosis is a trivial disease; the lack of a suitable animal model for EBV diseases except nonhuman primates; concern about the oncogenic potential of herpesvirus vaccines; and belief that the vaccine would not be commercially viable.

Progress is being made, albeit agonizingly slowly. Prophylactic EBV vaccines that have been in clinical trials will be discussed first followed by prospects for future vaccines. The mechanism of action of these vaccines is illustrated in Fig. 3.

Vaccinia construct expressing EBV membrane antigen gp220–340

The rationale to use EBV membrane antigen (that contains gp350) as the immunogen is that antibodies against EBV gp350 are closely related to neutralizing antibodies, which should prevent EBV from infecting B lymphocytes.[55]

Gu et al.,[56] from Beijing, China, published a phase 1 trial of gp220–340 vaccine in 1995. After immunogenicity was shown in

Table 1. EBV-related diseases: considerations for a vaccine trial

Disease target	Vaccine trial feasible	Pros	Cons	Primary population(s) affected	Annual incidence	Primary geographic region(s)
Burkitt lymphoma (endemic)	Yes	1. Short incubation period 2. Geographic target 3. High public health impact	1. Low incidence	Children and adolescents	0.18–0.46 per 100,000 males 0.14–0.26 per 100,000 females[77]	Eastern Africa
Infectious mononucleosis	Yes	1. Common 2. High participant acceptability 3. High public health impact	1. Target population healthy young adults	Adolescents and young adults	500 cases per 100,000 persons in United States[78]	Worldwide
Post-transplant lymphoproliferative disorder	Yes	1. Well-defined target population 2. Public health impact	1. Immunosuppressed population	Transplant recipients	224 cases per 100,000 persons (1st year post transplant)[79]	Worldwide
Hodgkin lymphoma	No	1. Public health impact	1. Low incidence 2. Long incubation 3. Unclear risk reduction	Adults (20–40 years and >55 years)	1 case per 100,000 persons[80]	Worldwide
Nasopharyngeal carcinoma	No	1. Geographic target	1. Low incidence 2. Long incubation	Children and adults	~1 case per 100,000 persons worldwide[81]	China Northern Africa Arctic
Multiple sclerosis	No	1. Well-defined target population	1. Long incubation	Adults (20–50 years)	3.6 cases per 100,000 women 2.0 cases per 100,000 men[82]	Northern hemisphere Distance from Equator
Chronic active EBV infection	No	1. Public health impact	1. Low incidence 2. Unclear risk reduction	Children and adults	Unknown; ~23.8 cases per year in Japan[51]	Japan United States

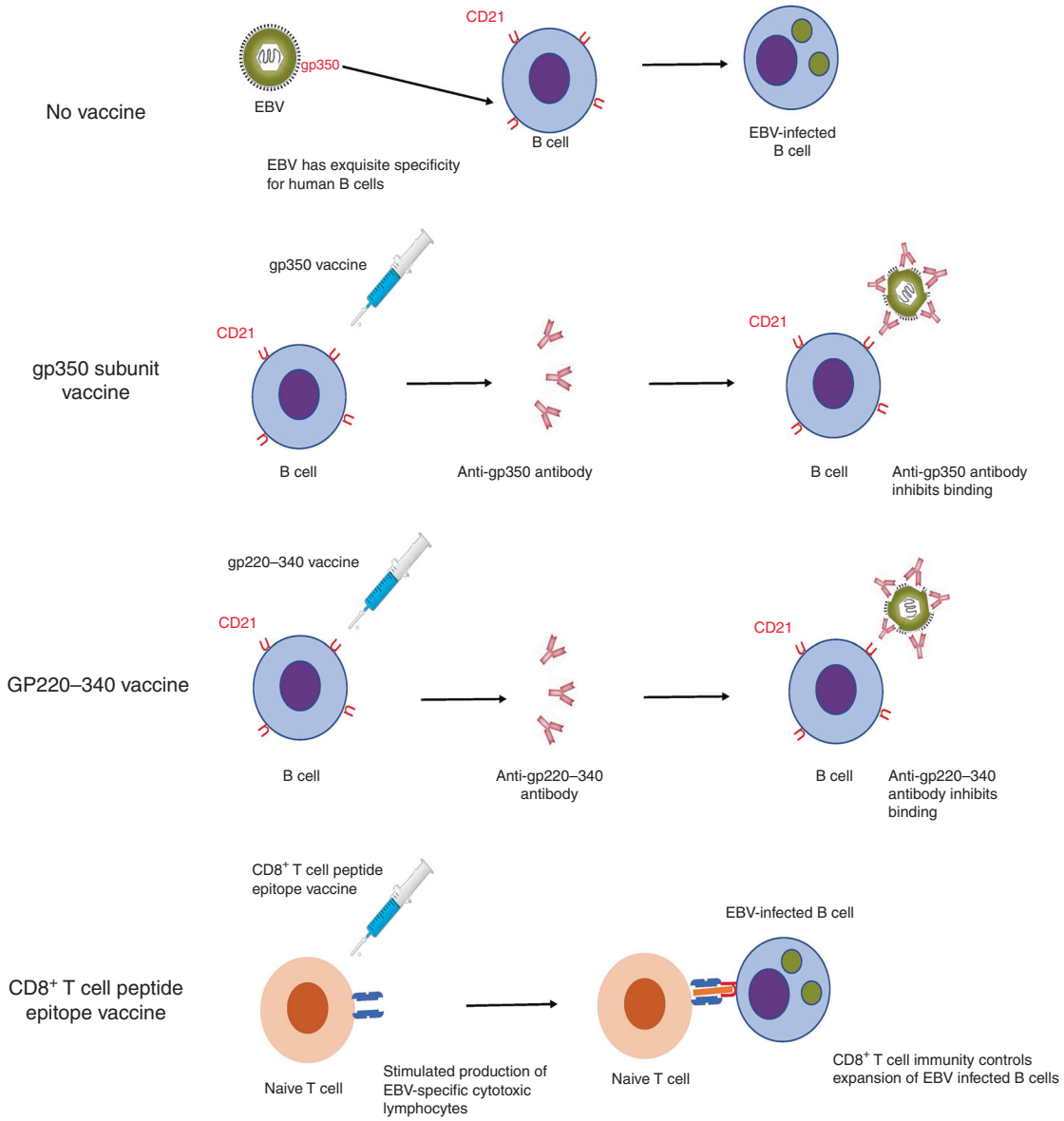

Fig. 3 Mechanism of action of EBV vaccines given to humans

rabbits, safety was established by vaccinating 11 adults and 6 children who had been previously infected by EBV. Next, the vaccine was given to 19 EBV-naive children 1.7–2.8 years of age. Nine children received a single dose of vaccine by scarification, whereas 10 children served as controls. The vaccine was immunogenic, eliciting antibodies against gp220–340 in eight of the nine vaccinees within 6 months. During 16 months of follow-up, three of nine vaccinees and all ten in the control group became infected with wild-type EBV as evidenced by development of antibodies against EBV VCA, which the vaccine did not contain. Thus, the vaccine showed the promise of efficacy, but no further work has been reported, probably because it contained live vaccinia, which is well known to be associated with adverse events.[57]

Subunit vaccines containing soluble EBV gp350

In 1999, Jackman et al.[58] produced a recombinant subunit EBV gp350 candidate vaccine in Chinese hamster ovary cells that elicited gp350 and neutralizing antibodies in rabbits. An EBV vaccine containing this or a very similar immunogen has subsequently been employed in four clinical trials.

A phase 1 study evaluated the safety and immunogenicity of a three-dose regimen of gp350 vaccine given intramuscularly.[59] EBV-naive and EBV-experienced participants 18 to 25 years of age were randomized to receive the vaccine adjuvanted with 3-O-desacyl-40-monophosphoryl lipid A and aluminum salt known as Adjuvant System 04 (AS04) or aluminum salt alone. A phase 1/2 study randomized EBV-naive volunteers 18 to 37 years old to receive unadjuvanted vaccine, vaccine adjuvanted with AS04, or vaccine adjuvanted only with aluminum salt. The immunogenicity data, which included measurement of gp350 and neutralizing antibodies, indicated that vaccine adjuvanted with AS04 or with aluminum salt was superior to non-adjuvanted vaccine.

The third trial was a phase 2, placebo-controlled, double-blind study evaluating safety, immunogenicity, and efficacy of recombinant gp350 vaccine adjuvanted with ASO4 in EBV-naive Belgians 16–25 years of age.[52] The vaccine 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 vaccine did not prevent infection: 13 (14%) of 90 vaccinees became infected versus 18 (20%) of 91 placebo recipients. However, it had a

significant effect on clinical disease. In the intention-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's exact test, one-sided).

Finally, a phase 1 study of recombinant gp350 vaccine with an aluminum hydroxide adjuvant was conducted in 16 pediatric kidney transplant candidates.[60] Subcutaneous doses of gp350 given three or four times over a total of 32 weeks were well tolerated. All 13 evaluable vaccinees mounted an anti-gp350 antibody response, but only 4 made neutralizing antibody. Because the study was too small and without a control group, vaccine efficacy could not be assessed. However, this phase 1 trial demonstrated that immunization of children awaiting kidney transplantation with EBV gp350 vaccine is feasible.

$CD8^+$ T cell peptide epitope vaccine

Another vaccine strategy is to control expansion of EBV-infected B cells by generating $CD8^+$ T cell immunity to EBNAs.[61] This phase 1 trial utilized an EBNA-3A peptide epitope (FLRGRAYGL) restricted by HLA B8[62] with tetanus toxoid formulated in a water-in-oil adjuvant as a source of T cell help.[63] EBV-naive individuals were vaccinated on a 2-month interval schedule. This strategy was effective at generating a peptide-specific $CD8^+$ T cell response in most individuals as measured by ex vivo peptide-specific interferon-γ production. Of the participants who subsequently acquired infection by wild-type EBV, infectious mononucleosis occurred in one of two placebo recipients as compared with zero of four in the vaccinated cohort, suggesting that this vaccine might prevent symptomatic EBV infection.

The general utility of epitope vaccines is limited, because they only target-specific HLA types. Nevertheless, epitope vaccines might be useful for preventing PTLD in transplant recipients whose HLA type is known prior to transplant.

Prospects for future prophylactic EBV vaccines

A gp350 subunit EBV vaccine, which is very similar to the one used in the Belgian phase 2 trial,[52] is being developed by our group in collaboration with an industrial partner.[64] Our gp350 vaccine is monomeric and the immunity it provides may not be sufficient to protect vaccinees from subsequent infection by wild-type EBV. Suggested improvements have been to deliver gp350 in a multimeric form or to include additional antigens.[65,66] Cui et al.[67] in the Snapper laboratory designed a tetrameric gp350 construct containing the first 470 amino acids of gp350 that induced much higher titers of gp350 and neutralizing antibodies in BALB/c mice as compared with its monomeric counterpart. In addition to tetrameric $gp350^{1-470}$, this group produced recombinant trimeric and monomeric EBV gH/gL heterodimeric proteins and a trimeric EBV gB protein.[68] These proteins were more immunogenic in male New Zealand white rabbits than monomeric $gp350^{1-470}$. The reason to put gB and gH/gL in a prophylactic EBV vaccine is that these glycoproteins are involved in fusion of EBV to B cells and epithelial cells.[69] Thus, theoretically, such a vaccine could prevent EBV from infecting B cells and epithelial cells.

Ogembo et al.[70] produced a subunit vaccine with a Newcastle disease virus (NDV) virus-like particle (VLP) platform containing EBV gp350/220 fused to NDV-fusion (F) protein. The chimeric protein EBV gp350/220-F was incorporated into the membrane of a VLP composed of the NDV matrix and nucleoprotein. The particles produced resembled EBV in shape and size. Vaccination of BALB/c mice resulted in the production of gp350 and neutralizing antibodies, which protected Raji cells from infection by a recombinant EBV construct.

The Cohen research group constructed a candidate EBV vaccine by fusing a portion of the ectodomain of gp350 to ferritin or encapsulin.[71] Nanoparticles were produced that contained 24 or 60 copies of EBV gp350. Vaccination of monkeys (*Cynomolgus macaques*) boosted EBV neutralizing antibodies. Vaccination of BALB/c mice with the nanoparticles induced neutralizing titers that were about 1000-fold higher than those obtained with soluble gp350. Importantly, vaccination with ferritin-gp350 nanoparticles protected mice from challenge with vaccinia virus expressing gp350, demonstrating that the antibodies produced were biologically active.

CONSIDERATIONS FOR DESIGNING FUTURE PROPHYLACTIC EBV VACCINES: CORRELATES OF IMMUNE PROTECTION

Although yet to be demonstrated in humans, recombinant subunit gp350 EBV vaccine adjuvanted with the synthetic toll-like receptor 4 agonist glucopyranosyl lipid A integrated into stable emulsion also elicited poly-functional anti-gp350 $CD4^+$ T cell responses in mice.[72]

A number of other immunogens have been proposed for inclusion in prophylactic EBV vaccines, including EBV glycoproteins (gp42, gH/gL, and gB), lytic proteins (Zta, Rta, BMLF1, BMRF1, BORF1, BcLf1, and BXLF1), and latent proteins (EBNA-2 and EBNA-LP).[66,73] The theory is that a vaccine containing multiple EBV antigens could provide broader protection from EBV infection than could be obtained by a monovalent vaccine.

Taking a rational approach to preventing viral entry, the Cohen research group reported that a vaccine containing the glycoproteins gH/gL or gH/gL/gp42 elicited potent B cell and epithelial cell neutralizing antibodies in BALB/c mice and nonhuman primates (*C. macaques*).[74] These antibodies also inhibited B cell and epithelial cell membrane fusion. The implication is that such vaccines could provide better protection by working at two steps in viral entry: attachment and fusion. A bonus is that the antibodies they elicit also protect epithelial cells from primary infection, which might lead to prevention of EBV-spurred malignancies, especially NPC.

CONCLUSION

A prophylactic EBV vaccine could reduce the burden of the many diseases EBV causes or spurs. Even if it only prevents or modifies infectious mononucleosis, that would be reason enough, in our opinion, to move forward with field trials of a candidate vaccine. The rationale to make this a pediatric vaccine administered before school entry is that the age at acquisition of primary EBV infection is very variable and dependent on race/ethnicity.[75,76] If the goal is to vaccinate a population that is at least 50% EBV naive, the age-specific EBV antibody prevalence of Minneapolis–St. Paul children tested in 2011–2012 indicated that non-Hispanic whites could be vaccinated in their teenage years, whereas non-Hispanic blacks would need to be vaccinated before 6 years of age, and multiracial children before age 10.[76] Because infectious mononucleosis is the result of primary EBV infection and is a risk factor for Hodgkin lymphoma and MS, it seems logical to vaccinate sooner than later.

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AUTHOR CONTRIBUTIONS

H.H.B. conceived, wrote, and edited the review. D.O.S. wrote and edited the review, and supplied Fig. 2. J.M.G.-G. wrote and edited the review, and produced Fig. 3.

ADDITIONAL INFORMATION

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