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Progress, prospects, and problems in Epstein-Barr virus vaccine development

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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.

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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 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 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 1–3 years of age were studied. Nine received the vaccine by scarification as a single dose containing 10^7 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

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 socioeconomics. $CD8^+$ 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.

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 16–25 [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.