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## Epstein-Barr Virus: An Important Vaccine Target for Cancer Prevention

Jeffrey I. Cohen<sup>1,\*</sup>, Anthony S. Fauci<sup>1</sup>, Harold Varmus<sup>2</sup>, and Gary J. Nabel<sup>1,3</sup>

<sup>1</sup>National Institute of Allergy and Infectious Diseases (NIAID), U.S. National Institutes of Health (NIH), Bethesda, MD 20892, USA

<sup>2</sup>National Cancer Institute, NIH, Bethesda, MD 20892, USA

<sup>3</sup>Vaccine Research Center, NIAID, NIH, Bethesda, MD 20892, USA

### Abstract

Participants at the February 2011 meeting at the U.S. National Institutes of Health on Epstein-Barr virus (EBV) vaccine research recommend that future clinical trials have two goals: prevention of infectious mononucleosis and EBV-associated cancers, facilitated by identification of disease-predictive surrogate markers.

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At a meeting held at the U.S. National Institutes of Health (NIH) on 1 February 2011, scientists from government, academia, and industry (1) assembled to discuss the merits of a vaccine for Epstein-Barr virus (EBV) and the opportunities and challenges for its development. Here, we highlight relevant clinical and epidemiological data and make recommendations for future EBV vaccine research and development.

### EBV EPIDEMIOLOGY

EBV is the principal etiological agent of infectious mononucleosis (IM) and is associated with several human cancers. There are at least 125,000 new cases of IM reported in the United States each year, and ~200,000 new cases of EBV-associated malignancies are reported each year worldwide (Table 1). Although effective vaccines are licensed for prevention of infection with hepatitis B virus and selected subtypes of human papillomaviruses (HPVs), which cause hepatocellular carcinoma and cervical carcinoma, respectively, a vaccine for EBV remains unavailable.

EBV infects more than 90% of the human population and is associated with a diverse array of diseases (2). Most infections occur during early childhood and are asymptomatic or cause nonspecific symptoms. However, in highly developed countries up to 50% of adolescents and young adults have not yet been infected, and nearly 50% of these susceptible individuals may develop IM if infected with EBV. IM presents with fever, swollen lymph nodes, sore throat, severe fatigue, and immune dysfunction. Although most persons with IM resolve their symptoms within 2 to 4 weeks, up to 10% can have fatigue that persists for 6 months or longer. Approximately 1% of persons have complications such as liver (hepatitis), brain (neurological), or blood (hematological) disease. IM is the major infectious cause of time lost for military recruits.

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\*Corresponding author. jcohen@niaid.nih.gov.

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EBV is associated with a wide range of cancers that vary in incidence in different parts of the world. In the United States, Hodgkin lymphoma, non-Hodgkin lymphoma, and nasopharyngeal carcinoma are the most common EBV-positive malignancies. In Southern China, the rate of EBV-associated nasopharyngeal carcinoma is 50 per 100,000 in men over the age of 50 years. EBV-positive Burkitt lymphoma is the most common childhood tumor in equatorial Africa and New Guinea. Gastric carcinomas are prevalent in Eastern Asia, Eastern Europe, and South America, with nearly 1 million new cases each year worldwide, and although only ~9% are EBV-positive, this cancer is the most common EBV-associated malignancy worldwide. Chemotherapy is effective for many EBV-associated malignancies, but up to 50% of survivors of all forms of Hodgkin lymphoma die from chemotherapy-associated complications, secondary malignancies, or disease relapse.

EBV-associated lymphomas occur in 1 to 20% of bone marrow and organ transplant recipients, depending on the type of transplant and the method of immunosuppression used, and is the second most common malignancy, after skin cancer, in organ transplant recipients. In persons infected with the human immunodeficiency virus (HIV), ~90% of Hodgkin and immunoblastic lymphomas and 50% of Burkitt lymphomas are EBV-positive. Although improved therapy for HIV has increased survival and reduced the prevalence of some tumor types in HIV-infected patients, the rates of EBV-positive Burkitt and Hodgkin lymphomas have not declined in this patient group and in the case of Hodgkin lymphoma, may actually be increasing. Other malignancies associated with EBV include certain non-Hodgkin lymphomas, T cell lymphomas, and smooth-muscle tumors.

Taken together, these epidemiological data show that prevention of these diseases through EBV vaccination would have a substantial public health and economic impact.

## EBV VACCINE STATUS

Most vaccine-development efforts to prevent EBV infection or related diseases have focused on the EBV glycoprotein gp350, which is the most abundant glycoprotein on the virus and on virus-infected cells and is the principal target of naturally occurring neutralizing antibodies. The largest EBV vaccine study in humans was a phase 2 placebo-controlled randomized clinical trial that involved 181 seronegative (uninfected) young adults who were vaccinated with recombinant gp350 that had been purified from Chinese hamster ovary cells that expressed the glycoprotein and delivered in an alum/monophosphoryl lipid A adjuvant. The vaccine did not protect against EBV infection [defined by the subsequent production of antibodies to nonvaccine EBV antigens (seroconversion) in the absence of IM], but did reduce by 78% the incidence of IM (defined by the manifestation of two or more EBV infection-related clinical symptoms and EBV seroconversion) (3). A gp350 vaccine is now under development by Medimmune, but the mechanism and efficacy of protection against EBV disease by a gp350-based vaccine is uncertain.

In a subsequent small clinical trial with patients who had chronic renal failure and were awaiting organ transplantation, administration of a lower dose of gp350 in alum resulted in poor immunogenicity, as measured by the presence of neutralizing antibodies to EBV (4). A vaccine that consisted of a peptide that corresponded to an EBV nuclear antigen (EBNA-3) expressed in tumor cells and tetanus toxoid in a water-oil adjuvant—designed to induce T cell-driven immunity to EBV infection and, conceivably, a potent T cell response against EBV-associated malignancies—induced virus-specific T cell responses in a small study of healthy volunteers (5) and requires further study.

Also in development are therapeutic vaccines to treat EBV-associated malignancies. The goal of these immunotherapies is to enhance T cell-mediated immunity to EBV proteins that are expressed in the tumor cells, especially EBV EBNA3s and latent membrane proteins

(LMPs). Indeed, a poxvirus that was engineered to express an EBNA1-LMP2 chimeric protein induced antigen-specific T cell responses in patients with nasopharyngeal carcinoma in phase I studies (6). Another approach for this disease is the infusion, into patients, of autologous EBV-specific cytotoxic T cells that have been expanded in vitro. In small clinical studies, this latter therapy induced complete responses, with resolution of lesions, in some patients with nasopharyngeal carcinoma and Hodgkin lymphoma. Although at present the preparation of EBV-specific cytotoxic T cells is very laborious, nonetheless these trial results strongly suggest that, for an EBV vaccine to be effective against virus-associated cancers, it must be able to induce a robust T cell-mediated immune response.

## ANIMAL MODELS FOR EBV RESEARCH

EBV infects only humans. Although mice that have been reconstituted with a human immune system do develop lymphoproliferation after infection with EBV, the immune systems of these animals are not identical to those of healthy humans. Vaccination of cotton top tamarin monkeys with gp350 protected the animals from malignant EBV-induced B cell lymphomas (7). However, use of this model system is limited because these animals are an endangered species and, because EBV is not a natural pathogen for them, very high titers of the challenge virus are needed to induce lymphoma.

An alternative approach is to use an EBV-related virus that infects nonhuman primates. Rhesus lymphocryptovirus is highly homologous to human EBV; indeed, every human EBV gene has a homolog in the rhesus virus. Oral inoculation of monkeys with rhesus lymphocryptovirus recapitulates many of the features of EBV in humans, including transient lymphoproliferation, virus shedding from the oropharynx, and viremia. Some animals develop virus-associated malignancies, and the incidence of these cancers is increased in immunosuppressed animals. Thus, rhesus lymphocryptovirus infection in monkeys might be a useful system for modeling candidate EBV vaccines.

## SURROGATE MARKERS FOR EBV-ASSOCIATED CANCERS

EBV vaccine development would be facilitated by the identification of biological “surrogate” markers that predict the development of protection from EBV infection or related malignancies. Thus, it is prudent to make use of previous lessons learned from vaccine studies for other cancer-associated viruses. Successful vaccination against HPV or HBV is known to induce high titers of infection-preventing antibodies. In this regard, there is strong evidence for progression from cervical dysplasia to cervical carcinoma in women infected with HPV types 16 and 18.

Similarly, for HBV there is a positive association between serum concentrations of hepatitis B surface antigen and development of hepatocellular carcinoma. These findings enabled vaccine trials that evaluated the ability of a vaccine to prevent acquisition of surrogate endpoints as markers for prevention of virus-associated cancer. In contrast, although elevated serum concentrations of immunoglobulin A-type antibodies to the EBV virus capsid antigen (VCA) and EBV genomic DNA are observed before the development of nasopharyngeal carcinoma and posttransplantation lymphoproliferative disease, respectively, surrogate biomarkers that predict most EBV-associated malignancies have not yet been identified.

## IMPLICATIONS AND RECOMMENDATIONS

Several clinical observations suggest that an EBV vaccine might protect against IM and reduce EBV-associated cancers without achieving sterilizing immunity that completely prevents EBV infection. First, primary asymptomatic EBV infection protects against

subsequent development of IM despite repeated exposures to EBV. Second, a history of IM is associated with a 3.4-fold increased risk of EBV-related Hodgkin lymphoma (8). Thus, a vaccine that reduces the incidence of IM might also reduce that of Hodgkin lymphoma without preventing EBV infection. Third, because serum EBV DNA concentrations increase before the onset of lymphoma after transplantation and are elevated at the onset of nasopharyngeal carcinoma, an EBV vaccine that allows the immune system to control the viral DNA load in the blood might reduce the occurrence of these EBV-associated malignancies even if the vaccine does not prevent viral infection.

On the basis of these observations and the promising results from the phase 2 gp350 vaccine trial in healthy humans, we recommend that a phase 3 trial of an EBV vaccine be designed and conducted with the goal of licensure for the prevention of IM and that blood EBV DNA concentrations be measured in placebo and EBV-vaccinated trial participants who subsequently become infected with EBV to determine whether the vaccine also reduces the viral load after natural infection. Specific trials with the gp350 or other potentially improved vaccines (for example, those that induce EBV-neutralizing antibodies and T cell-mediated immunity to EBV proteins expressed in tumor cells) could be undertaken nationally and internationally to address their efficacy in preventing EBV-associated malignancies.

Furthermore, we propose the following priorities for future research:

- Identification of surrogate markers that predict the development of EBV-related malignancies in vaccine studies, and biomarkers that permit screening for early-stage EBV-associated malignancies.
- Determination of immune correlates of protection against EBV infection and disease in animal models and in humans.
- Definition of a goal for an EBV vaccine and criteria for licensure: to prevent disease rather than infection.
- Planning of additional epidemiological studies designed to predict the likelihood of benefit of a specific EBV vaccine and development of a strategy to determine an EBV vaccine's efficacy in preventing human cancers.
- Establishment of mechanisms to facilitate collaborations among academic and industry scientists and clinicians for further development and improvement of the gp350 vaccine for IM and the development of second-generation EBV vaccines.

## References and Notes

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**Table 1****EBV by the numbers.** Estimated new cases of EBV-associated cancers worldwide per year (9, 10).

Cancer	Number of cases	Number of cases attributable to EBV
Burkitt lymphoma		
Developed countries	400	100
Less-developed countries	7800	6600
Gastric carcinoma	933,900	84,050
Hodgkin lymphoma	62,400	28,600
Nasopharyngeal carcinoma	80,000	78,100
Total		197,450