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

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(See the editorial commentary by Balfour, on pages 1724–6.)

Background.   To date, there is no commercially available vaccine to prevent infectious mononucleosis, a disease frequently induced by Epstein-Barr virus (EBV) infection in adolescents or adults devoid of preexisting immunity to the virus.

Methods.   A total of 181 EBV-seronegative, healthy, young adult volunteers were randomized in a double-blind fashion to receive either placebo or a recombinant EBV subunit glycoprotein 350 (gp350)/aluminum hydroxide and 3-*O*-desacyl-4'-monophosphoryl lipid A (AS04) candidate vaccine in a 3-dose regimen.

Results.   The vaccine had demonstrable efficacy (mean efficacy rate, 78.0% [95% confidence interval {CI}, 1.0%–96.0%]) in preventing the development of infectious mononucleosis induced by EBV infection, but it had no efficacy in preventing asymptomatic EBV infection. One month after receipt of the final dose of gp350 vaccine, 98.7% of subjects showed seroconversion to anti-gp350 antibodies (95% CI, 85.5%–97.9%), and they remained anti-gp350 antibody positive for >18 months. Furthermore, there were no concerns regarding the safety or reactogenicity of the gp350/AS04 vaccine.

Conclusion.   These data support the clinical feasibility of using an EBV vaccine to prevent infectious mononucleosis.

Trial registration.   ClinicalTrials.gov identifier: NCT00430534.

Epstein-Barr virus (EBV) is a common human γ-herpesvirus that is prevalent in human populations worldwide [1]. EBV infection is usually asymptomatic in children. However, 30%–40% of adolescents (age, ≥15 years) who contract the virus will develop infectious mononucleosis [2]. The acute phase of illness is characterized by a triad of symptoms—cervical lymphadenopathy, fever, and pharyngitis—followed by a general feeling of fatigue and malaise, which may last for several months. Infectious mononucleosis occasionally may lead to serious complications, including fulminant hepatic failure, splenic rupture, and hematologic disorders [3].

After initial infection, the host becomes a lifelong carrier of EBV. In rare instances, EBV has been associated with several oncogenic presentations, including Burkitt lymphoma, posttransplantation lymphoproliferative disorders, lymphoma in HIV-infected patients, and nasopharyngeal carcinoma [1, 4, 5]. Furthermore, it has

been suggested that EBV is associated with chronic fatigue syndrome [6], Hodgkin disease [7], and multiple sclerosis [8].

At present, there is no commercially available vaccine to prevent EBV-associated disease, although a number of developmental formulations are under investigation. Glycoprotein 350 (gp350) is a viral antigen expressed on the EBV capsid that facilitates entry of EBV into B cells by attaching to the CD21 antigen receptors on their surface. It is targeted by the immune system after natural infection [9–11] and has therefore attracted considerable interest as a potential vaccine candidate. In the early 1990s, a gp350-based vaccine was tested in children [12]. Despite the fact that findings regarding anti-gp350 seroconversion and efficacy were encouraging, this vaccine did not seem to be developed further, and the findings were not confirmed.

In our early studies of an anti-gp350 EBV vaccine, we showed a recombinant gp350 subunit/AS04 preparation to be generally well tolerated and immunogenic [13]. After noting such encouraging results, we conducted this phase 2 trial to examine the immunogenicity and safety of the recombinant viral gp350/AS04 vaccine and measure its efficacy in preventing infectious mononucleosis in healthy young adults.

MATERIALS AND METHODS

Vaccine. The gp350/AS04 and placebo vaccines were formulated by GlaxoSmithKline Biologicals (Rixensart, Belgium). Each dose of vaccine contained 50 μg of gp350 and AS04 Adjuvant System (0.5 mg of aluminum hydroxide and 50 μg of 3-O-desacyl-4'-monophosphoryl lipid A) in a 0.5-mL volume. Each dose of placebo contained 0.5 mg of aluminum hydroxide in a 0.5-mL volume. Recombinant gp350 was purified from the supernatant of Chinese hamster ovary cells expressing a gp350/220 gene construct with splice-site mutations that prevent population of the gp220 isoform (MSTOP gp350) [9]. Cells were cultured in suspension in the absence of fetal bovine serum.

Subjects. The study was performed at 5 locations across Belgium (Antwerp, Brussels, Charleroi, Leuven, and Liège) between October 2001 and December 2003. Of the 2145 subjects who underwent screening, a total of 181 volunteers who were 16–25 years of age and were found to be negative for serological markers of EBV infection (i.e., had negative antibody titers according to anti–viral capsid antigen [VCA] immunoglobulin [Ig] G and IgM ELISA [see the "Serological analysis" subsection below]) were enrolled. All subjects were healthy, as determined by medical examination, and did not meet any of the following exclusion criteria: clinical signs of acute illness or fever; history of infectious mononucleosis; major congenital defects or serious chronic illness; history of neurological disorders or seizures; injection drug use; sensitivity to vaccine components; immunosuppression, including that due to chronic treatment with immunosuppressive drugs (such as corticosteroids) or to immunosuppressive or immunodeficient conditions; simultaneous

participation in any other clinical trial; simultaneous receipt of any other vaccine(s); receipt of immunoglobulins during the study or during the 3 months before administration of the first vaccine dose; and pregnancy or lactation. Approval by the local ethics committee was obtained from the study centers, and each subject provided written, informed consent. Female volunteers agreed to use appropriate contraception during the first 7 months of the study, and urine pregnancy testing was performed before each vaccine injection.

Study design. Volunteers were randomized in a double-blind fashion to receive 3 doses of either vaccine or placebo in a 1:1 ratio. Both types of doses were administered intramuscularly into the deltoid at 0, 1, and 5 months. Blood samples were obtained at the time of medical visits at months 0, 1, 5, 6, and 19 of the study, for determination of anti-VCA and anti-gp350 antibody levels.

Vaccine efficacy was assessed in all subjects over the 18 months of follow-up after administration of the second injection (up to month 19 of the study). Subjects were briefed on infectious mononucleosis symptoms and received an information sheet that recapitulated these symptoms. They were instructed to record and report any suspicion of infectious mononucleosis to the investigator, and they were reminded every month to do so. Each time a case of infectious mononucleosis was suspected, an additional medical visit was scheduled. Disease symptoms and date of onset were recorded at the clinic, and blood samples were collected for laboratory analysis. If infectious mononucleosis was confirmed, additional monthly visits were arranged until recovery.

Serological analysis. Assessment of EBV serological status before vaccination, as well as monitoring of EBV infection during the course of the trial, was performed at Henogen (Gosselies, Belgium). Antibodies to EBV nonvaccine antigens were assessed using a recombinant anti-VCA IgG and IgM ELISA (Biotest). Negative ELISA results noted at baseline and positive ELISA results noted between months 1 and 18 were confirmed using anti-VCA immunofluorescence against IgG and IgM (Euroimmun). All results falling within ±20% of the ELISA cutoff value, as defined by the manufacturer, were confirmed using immunofluorescence.

During the trial, anti-gp350 antibody titers were measured by use of a gp350 ELISA, which was developed and validated at Henogen. This method was used along with a competition ELISA, based on the 72A1 monoclonal antibody, to measure EBV-neutralizing activity [14].

Sample analysis, including hematologic and biochemical analysis and evaluation of heterophile, cytomegalovirus (CMV), and toxoplasma antibodies, was performed in a blinded fashion by study center laboratories.

Case definitions of infectious mononucleosis and EBV infection. Suspected cases of infectious mononucleosis were reviewed by an external EBV expert and at data review meetings,

Table 1. Distribution of cases of infectious mononucleosis and Epstein-Barr virus infection among vaccine and placebo recipients.

Case, presentation category	ATP population		ITT population	
	Given placebo ($n = 90$)	Given gp350/AS04 vaccine ($n = 86$)	Given placebo ($n = 91$)	Given gp350/AS04 vaccine ($n = 90$)
Infectious mononucleosis	8	2	9	2[a]
Definite	8	2	8	2
Probable	0	0	1	0
Asymptomatic infection	9	11	9	11
Total	17	13	18	13

NOTE. Data are no. of subjects. AS04, aluminum hydroxide and 3-*O*-desacyl-4'-monophosphoryl lipid A; ATP, according to protocol; gp350, glycoprotein 350; ITT, intention to treat.

[a] The difference between the vaccine and placebo group reaches statistical significance ($\alpha = 0.05$, by 1-sided Fisher's exact test).

before the trial was unblinded. Case presentation categories included "definite," "probable," or "possible" infectious mononucleosis, as well as asymptomatic EBV infection.

Definite cases of infectious mononucleosis were defined as cases occurring in subjects who, during the study period, showed seroconversion to EBV nonvaccine antigens (IgM or IgG antibodies, as assessed by VCA ELISA), accompanied by $\geqslant 2$ clinical symptoms related to EBV (e.g., fatigue, sore throat, painful lymph nodes, fever [body temperature, $>37°C$], excessive sleeping, headache, sore muscles, nausea, sore joints, cough, or rash), but who did not demonstrate seroconversion to CMV or toxoplasma antigens. Suspected cases occurring in subjects who showed no seroconversion to VCA were defined as definite noncases of infectious mononucleosis.

"Probable" and "possible" clinical presentations were defined before the study began, to identify subjects who showed seroconversion to EBV nonvaccine antigens but for whom incomplete data were available or subjects whose clinical presentations were unclear and for whom an etiology other than EBV was suspected, respectively. EBV infection, defined by seroconversion to nonvaccine EBV antigens (IgM or IgG antibodies) that was not accompanied by symptoms of infectious mononucleosis, was recorded as asymptomatic EBV infection.

Data analysis. The trial enrolled 181 healthy adult volunteers. Under the assumption of a true vaccine efficacy rate of 90% and an infectious mononucleosis attack rate of 7% per year, Fisher's exact test with a 1-sided significance of $\alpha = 0.05$ would have 80% power to detect a difference in the incidence of infectious mononucleosis, with 83 subjects per group. A distribution of 10 cases in the placebo group and up to 3 cases in the vaccine group would reach levels of statistical significance.

The primary end point was the incidence of infectious mononucleosis in vaccine and placebo groups 18 months after administration of the second vaccine dose. The according-to-protocol (ATP) population was used for primary analyses of vaccine efficacy, which was defined as $1 -$ (attack rate in the vaccine group/attack rate in the placebo group). A 2-sided 95% confidence interval (CI) around the vaccine efficacy rate (estimated as $1 -$

relative risk [RR]) was computed (using the Mantel-Haenszel CI for the RR). Odds ratios (ORs) for contracting infectious mononucleosis were calculated using a 1-sided Fisher's exact test ($\alpha = 0.05$), with and without stratification for center effects.

Secondary end points included the efficacy of the vaccine in preventing primary EBV infection (symptomatic and asymptomatic cases) and the immunogenicity of the vaccine in terms of humoral response, as well as safety and reactogenicity. The vaccine was differentiated from placebo by use of a 1-sided Fisher's exact test. The main analyses of safety and reactogenicity were performed using the "intention-to-treat" (ITT) population. Attack rates were calculated as the number of cases divided by the number of subjects, expressed as a percentage.

RESULTS

Demographic characteristics. A total of 181 volunteers, all of whom were found to be negative for serological markers of EBV infection, were randomized to receive placebo or gp350/AS04 vaccine. A total of 90 of 91 participants in the placebo group and 88 of 90 patients in the vaccine group completed the study (until month 19).

The study groups were well balanced in terms of age, with a mean age of 20.5 years for subjects in the placebo group and 20.6 years for subjects in the vaccine group. Both groups were similarly well balanced in terms of distributions of sex (percentage of subjects who were male, 53.8% of the placebo group and 51.1% of the vaccine group) and race (percentage of subjects who were white, 96.7% of the placebo group and 97.8% of the vaccine group).

Efficacy. Two definite cases of infectious mononucleosis were confirmed in subjects receiving gp350 vaccine, and 8 definite cases were confirmed in subjects in the placebo group, when efficacy analysis was ATP based (table 1). This distribution did not reach statistical significance ($P = .06$; $\alpha = 0.05$, by 1-sided Fisher's exact test). In ITT-based efficacy analysis, infectious mononucleosis cases were distributed as follows: 9 cases (1 probable and 8 definite) were found in the placebo group and 2 cases
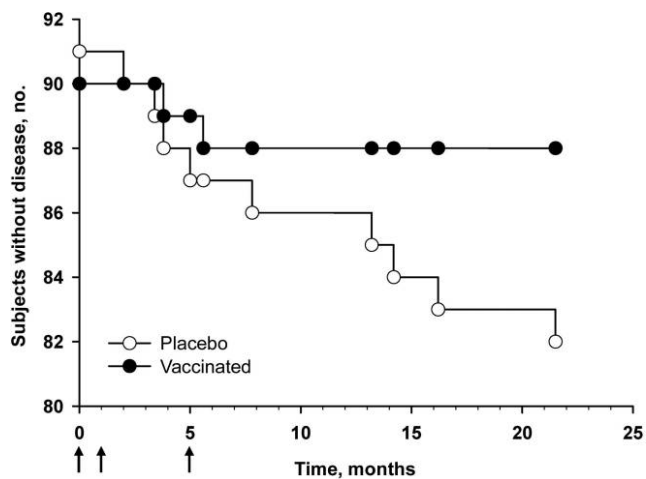
Figure 1. Timing of occurrence of infectious mononucleosis in vaccine and placebo recipients (an intention-to-treat cohort). Arrows denote the times of vaccination. After completion of the study, 1 additional case of infectious mononucleosis was reported in the placebo group.

(both definite) were found in the vaccine group ($P = .03$; $\alpha = 0.05$, by 1-sided Fisher's exact test) (table 1), representing attack rates of 2.22% per 1.5 years (95% CI, 0.27%–7.80%) for the vaccine group and 9.89% (95% CI, 4.62%–17.85%) for the placebo group. Of the 2 cases that occurred in vaccinated subjects, 1 was associated with very limited disease symptoms (low-grade sore throat and painful lymph nodes) and, consequently, was on the outer limits of criteria for disease confirmation. In addition, because this case occurred 3 months after the first vaccine injection (figure 1), and because an incubation period of several weeks can be expected, this individual may have been infected before he received the second vaccine dose. The second case occurred in an individual who presented with infectious mononucleosis 5 months after the first vaccine injection and who seemed not to be highly responsive to the vaccine on the basis of the anti-gp350 titer noted at that time (6.5 U vs. a mean titer of 26.96 U [95% CI, 21.35–34.06 U] for the vaccine group), which might explain why the individual contracted the disease. The probable case that occurred in the placebo group could not be confirmed because clinical symptoms were not adequately reported to the investigator.

An additional case of infectious mononucleosis was reported in a placebo recipient 21 months after the second vaccine injection was administered and before study unblinding took place; however, it was reported too late to be incorporated into the database.

Vaccine efficacy, which was measured by the ability of the vaccine to prevent infectious mononucleosis, was 78.0% (95% CI, 1%–96%) when the ITT population was used for analysis. The OR for contracting infectious mononucleosis was 0.11 in the placebo group and 0.023 in the vaccine group. The estimated OR for the 2 groups was 4.80 (1-sided 95% CI, >1.30), indicat-

ing that the odds of developing a case of infectious mononucleosis were 4.8 times more likely in the placebo group than in the vaccine group. When the data were stratified for center effects, the estimated common OR was 5.10 (1-sided 95% CI, >1.34). The difference between the incidence of asymptomatic EBV infections occurring in volunteers receiving vaccine or placebo (11 vs. 9 cases, respectively) did not reach statistical significance.

Immunogenicity. Six months after the first dose of gp350 vaccine was administered (i.e., 1 month after the third dose), 98.7% (95% CI, 85.5%–97.9%) of subjects who had not already demonstrated conversion for anti-VCA antibodies showed sero-conversion for anti-gp350 antibodies, as measured by gp350 ELISA (cutoff, 10 U/mL). Geometric mean titers were 356.23 U (95% CI, 276.22–459.42 U) for the gp350 vaccine group and 2.83 U (95% CI, 2.54–3.14 U) for the placebo group. In comparison, the 9 subjects in the placebo group who developed asymptomatic EBV infection during the course of the trial had gp350 ELISA titers of 11.9–84.6 U at the fifth medical visit (14 months after the third dose). Competition ELISA values peaked at 6 months after the first injection, with 69.86% (95% CI, 58.00%–80.06%) of subjects showing seroconversion at that point in time.

Safety. Study medication was well tolerated, and no subject discontinued medication for reasons of safety or reactogenicity. After each of 3 doses, the mean percentage of patients presenting with adverse events was slightly higher in the vaccine group than in the placebo group: local pain (85.7%, 73.6%, and 73.6% vs. 63.7%, 52.6%, and 58.2%, respectively), redness (20.9%, 26.4%, and 27.5% vs. 15.4%, 14.3%, and 13.2%, respectively) and swelling (11.0%, 16.5%, and 20.9% vs. 5.5%, 8.8%, and 7.7%, respectively). Systemic adverse events occurred less frequently in the vaccine group, with no statistical difference noted between gp350 vaccine and placebo. The most commonly solicited general symptoms were fatigue and headache. One case of appendicitis was reported in a subject receiving placebo, but this event was not considered to be related to vaccination.

DISCUSSION

The primary objective of the present phase 2 study was to estimate the efficacy of a recombinant gp350 vaccine in protecting EBV-seronegative subjects against infectious mononucleosis during the study period. Although the frequency of infectious mononucleosis was not statistically different between both groups in the ATP analysis, the vaccine had demonstrable efficacy (mean efficacy rate, 78.0% [95% CI, 1.0%–96.0%]) when the incidence of infectious mononucleosis in the ITT population was assessed. The number of EBV infections was limited, and the 95% CI of the calculated efficacy was 1.0%–96.0% because of the small study population. The results of the present study suggest that 3 doses of vaccine are necessary before full protection is afforded. Indeed, no case of infectious mononucleosis was de-

clared in the vaccine group after completion of the vaccination schedule, whereas cases still occurred regularly in the placebo group. Moreover, only after administration of the third vaccine dose was the level of induced anti-gp350 antibodies found to be higher than that resulting from natural infection.

The gp350/AS04 vaccine that we used largely prevented the development of infectious mononucleosis after EBV infection, but it did not prevent asymptomatic EBV infection. It is noteworthy that the total number of EBV infections (symptomatic and asymptomatic cases) was marginally reduced in the vaccine group compared with the placebo group (13 vs. 18 cases, respectively). It would be of considerable interest to see whether this trend develops further by evaluating a larger number of subjects. It would also be interesting, in such a larger panel, to study the mechanisms of protection that are induced by vaccination—in particular, the effect on the EBV load after infection, because it has been suggested that a reduction in this parameter correlates with protection against infectious mononucleosis [15].

It is unclear whether the gp350 EBV vaccine can prevent other potential complications of EBV infection, such as posttransplantation lymphoproliferative disorder, which may progress to true B lymphoma (Burkitt or Hodgkin lymphoma) [5, 7, 16]. A vaccine that could prevent EBV infection may be of considerable clinical benefit, especially for patients undergoing organ transplantation or those with immunosuppressive conditions.

This study was not designed to assess the duration of protection of the gp350/AS04 vaccine. However, as illustrated in figure 1, the time of occurrence of infectious mononucleosis in vaccine recipients and placebo recipients in the trials suggests that the duration of protection after vaccination may exceed 18 months.

This is the first demonstration of the considerable efficacy of a vaccine to prevent infectious mononucleosis after EBV infection in a small study. The gp350 vaccine formulation was well tolerated, and there were no specific concerns related to safety. Together, these findings show promise for further exploration of the use of this gp350/AS04 vaccine in the prevention of infectious mononucleosis.

Acknowledgments

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