

Dose-Related Safety and Immunogenicity of a Trivalent Baculovirus-Expressed Influenza-Virus Hemagglutinin Vaccine in Elderly Adults

John J. Treanor,¹ Gilbert M. Schiff,² Robert B. Couch,³ Thomas R. Cate,³ Rebecca C. Brady,² C. Mhorag Hay,¹ Mark Wolff,⁴ Dewei She,⁴ and Manon M. J. Cox⁵

¹University of Rochester, Rochester, New York; ²Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio; ³Baylor College of Medicine, Houston, Texas; ⁴EMMES Corporation, Rockville, Maryland; ⁵Protein Sciences Corporation, Meriden, Connecticut

Background. Influenza-virus hemagglutinin (HA) protein expressed in insect cells by recombinant baculovirus is a candidate influenza vaccine.

Methods. In a randomized, double-blind trial conducted in 399 adults ≥ 65 years of age, the efficacy of trivalent inactivated influenza vaccine (TIV) licensed for intramuscular injection was compared with that of trivalent baculovirus-expressed HA vaccine administered at doses of 15 μg , 45 μg , or 135 μg of each HA.

Results. Compared with TIV, baculovirus-expressed HA vaccine was safe and induced better serum antibody responses to the H3 component when administered at doses of 45 μg or 135 μg of each HA.

Conclusions. Baculovirus-expressed HA is a safe and immunogenic influenza vaccine in elderly adults.

All currently licensed influenza vaccines are generated in embryonated hen's eggs. Several well-recognized disadvantages to the use of such eggs as the substrate for influenza-vaccine production include the potential vulnerability of the supply of eggs, the long lead time required to scale up egg production, and the need to adapt new variants for high-yield growth in eggs, a process that can be time consuming and is not always successful. In addition, growth in eggs can result in selection of receptor variants that may not be optimal for protection against circulating strains [1].

An alternative method for production of influenza vaccine is expression of the main vaccine antigen, hemagglutinin (HA), by recombinant-DNA techniques. In the present study, we evaluated rHA0, an HA produced

in insect cells by a recombinant baculovirus. This alternative avoids dependence on eggs, and the efficient protein expression under the control of the baculovirus polyhedrin promoter facilitates the use of high doses of HA in the vaccine. Monovalent and bivalent baculovirus-derived influenza vaccines have been evaluated in both young and elderly adults and are well tolerated and immunogenic [2–5]. The purpose of the present study was to evaluate the tolerability of higher doses of a trivalent formulation of baculovirus-expressed HA vaccine in an elderly population and to determine whether the use of high doses would result in better immune responses than are seen after administration of conventional inactivated-influenza vaccine.

SUBJECTS, MATERIALS, AND METHODS

Clinical study design. The study was conducted as a randomized, prospective, observer- and subject-blinded trial. Subjects were community-dwelling, medically stable adults ≥ 65 years of age. Subjects with immunosuppressive illnesses were excluded from participation, but other high-risk conditions could be present as long as they were considered to be stable at the time of immunization. Informed consent was obtained from all subjects, and the human-experimentation guidelines of the US Department of Health and Human Services

Received 9 September 2005; accepted 9 December 2005; electronically published 28 March 2006.

Potential conflicts of interest: M.M.J.C. is an employee of Protein Sciences Corporation. None of the other authors have a commercial or other association that might pose a conflict of interest.

Financial support: National Institute of Allergy and Infectious Diseases (contracts N01 AI 25465, N01 AI 25459, and N01 AI 25460); National Center for Research Resources (contract M01 RR00044).

Reprints or correspondence: Dr. John J. Treanor, Infectious Diseases Div., University of Rochester Medical Center, 601 Elmwood Ave., Rm. 3-6309, Rochester, NY 14642 (john_treanor@urmc.rochester.edu).

The Journal of Infectious Diseases 2006;193:000–000

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and of the participating institutions were followed throughout the study.

Subjects were randomized to receive either trivalent subvirion vaccine or a trivalent rHA0 vaccine containing either 15 μg , 45 μg , or 135 μg of each rHA0. Because virtually all subjects had previously received influenza vaccine, randomization was not stratified on the basis of influenza-vaccination history.

The study was conducted in 2 stages. In the first stage, 80 subjects were randomly assigned to the 4 dose groups, so that each group contained 20 subjects; after rates of side effects in subjects enrolled in the first stage had been evaluated by the safety-monitoring committee, the remaining subjects were enrolled in the study.

For 7 days after vaccination, subjects maintained a symptom diary card on which they recorded local and systemic symptoms, and they returned to the clinic on days 2 and 7 after vaccination, for review of their symptoms. Serum samples were obtained from all subjects both before vaccination and 28 days after vaccination. In addition, complete blood count and levels of serum creatinine, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and creatinine phosphokinase were measured in subjects enrolled in the first stage of the study, both at enrollment and on day 7 after vaccination.

Vaccine. The HA genes of the 3 influenza viruses—A/Panama/2007/99 (H3N2), A/New Caledonia/20/99 (H1N1), and B/Hong Kong/330/2001—represented in the vaccine were independently cloned into the plasmid expression vector pPSC12. The PSC12 plasmid contains the AcNPV baculovirus polyhedrin promoter, the baculovirus 61K signal peptide, and flanking baculovirus DNA derived from the *EcoRI*-I fragment of AcNPV. After confirmation of the correct sequence, the DNA sequences were inserted into AcNPV by homologous recombination. Recombinant viruses containing the respective HA genes were then used to express the HAs in the high-yield SF9-derived insect cell line *expresSF+*, in serum-free conditions.

The HA proteins expressed under these conditions form trimeric structures as revealed by electron microscopy and are not cleaved in insect cells in the absence of exogenously added proteases; therefore, they are referred to as “rHA0.” The proteins were purified by a combination of anion-exchange and lectin chromatography to >95% purity as assessed by protein-gel electrophoresis. The vaccine was formulated at final concentrations of either 15 μg , 45 μg , or 135 μg of each rHA0 per 0.5-mL dose (resulting in total doses of 45 μg , 135 μg , and 405 μg of rHA0, respectively) in sodium phosphate buffer with 0.01% Tween 20 (pH 7.4). The licensed trivalent inactivated influenza vaccine (TIV) used as a control in this study was egg-grown, commercially available subvirion vaccine (Fluzone; Sanofi-Pasteur) formulated to contain 15 μg of each HA component.

The amounts of HA included in rHA0 and TIV were determined by 2 methods: single radial immunodiffusion (SRID), in

the case of TIV, and modified Lowry assay of total protein content, in the case of the rHA0 vaccine. Subsequent tests showed significant discrepancies between these 2 assay methods when used to estimate the content of the rHA0 vaccine preparations. Although the amount of A/Panama/2007/99 (H3N2) rHA0 (H3 component) estimated by SRID was equivalent to that determined by Lowry assay, the amount of A/New Caledonia/20/99 (H1N1) rHA0 (H1 component) measured by SRID was only 30% of that determined by Lowry assay, and the amount of the B/Hong Kong/330/2001 rHA0 was 80% of that determined by Lowry assay; thus, rHA0 doses of 135 μg , 45 μg , and 15 μg corresponded to 40.5 μg , 13.5 μg , and 4.5 μg , respectively, as determined by SRID, for the H1 component and to 127 μg , 37 μg , and 12 μg , respectively, as determined by SRID, for the B component. In this report, the rHA0-dose groups are referred to as originally intended—that is, as the 15- μg , 45- μg , and 135- μg (of each rHA0)-dose groups.

Immunogenicity analysis. Serum levels of antibody against each of the 3 vaccine strains were measured independently of the sponsor and blinded as to vaccine assignment, by microtiter hemagglutination inhibition (HAI) as described elsewhere [6]. Serum samples were treated with receptor-destroying enzyme (Senka) before use and were tested at an initial dilution of 1:4. Antigens were egg-grown pools of the test viruses, and the B antigen was ether extracted. Also, a randomly chosen subset of serum samples were tested for neutralizing antibody (NtA) to A/Panama/2007/99 (H3N2), by microtiter neutralization as described elsewhere [2].

Statistical methods. In the present study, the predefined primary efficacy end point was the proportion of subjects achieving a postvaccination serum HAI titer of $\geq 1:128$ against the H3 component of the vaccine. This end point was chosen both because H3N2 influenza viruses repeatedly have been shown to cause the majority of influenza-associated hospitalizations and deaths [7, 8] and because a large proportion of repeatedly vaccinated elderly adults would be expected to have prevaccination titers at or above the traditional 1:32 end point. The choice of the specific titer to use as the primary end point was made before the start of the study, but it was arbitrary and was not based on data showing that the titer is specifically associated with protection in elderly adults.

The working hypothesis for this trial was that a 20% improvement in the proportion of individuals achieving this end point would represent a clinically significant improvement in vaccine performance. Inclusion of 100 subjects per dose group gave 80% power to detect a $\geq 20\%$ difference in rates against the H3 component, by a 2-sided uncorrected χ^2 test and an assumed dropout rate of $\leq 3\%$.

Comparisons of proportions were calculated by StatXact using exact Pearson's χ^2 test and were not adjusted for multiple comparisons. Pre- and postvaccination geometric mean titers

Table 1. Local and systemic side effects ≤ 7 days after vaccination.

Dose group	Subjects, no. ^a								
	Total	Experiencing symptoms after vaccination ^b							
		Local			Systemic				
		Pain	Tenderness	Swelling	Headache	Malaise	Myalgia	Nausea	Fever
TIV	99	6 (1)	29 (1)	3 (0)	10 (5)	7 (2)	8 (1)	2 (1)	2 (1)
rHA0									
15 μ g	99	11 (0)	14 (0)	1 (0)	15 (1)	13 (6) ^c	11 (5) ^c	6 (3) ^c	3 (1) ^c
45 μ g	100	15 (3)	20 (0)	0 (0)	14 (6)	14 (4) ^c	8 (2) ^c	5 (2)	4 (1)
135 μ g	101	19 (1) ^c	29 (1)	11 (3)	15 (3) ^d	15 (3) ^d	15 (3) ^d	5 (1)	4 (1) ^c

NOTE. rHA0, hemagglutinin expressed by recombinant virus in insect cells; TIV, trivalent inactivated influenza vaccine.

^a Numbers in parentheses are number of subjects with moderate or severe symptoms.

^b Severity of symptoms was graded as mild (noticeable but not interfering with activities), moderate (some interference with activities), or severe (prevents normal daily activities). Swelling of >50 mm diameter was considered to be moderate, >100 mm diameter severe. Fever was defined as an oral temperature of $\geq 37.5^\circ\text{C}$, moderate as $\geq 38.5^\circ\text{C}$, and severe as $\geq 39.5^\circ\text{C}$.

^c One event reported as severe.

^d Two events reported as severe.

(GMTs) of antibody were compared by analysis of variance (ANOVA).

RESULTS

Tolerability. A total of 399 subjects were enrolled. The mean age of participants was 72 years (range, 65–90 years); 96% of the subjects identified themselves as white, and 51% were female. Chronic but stable medical conditions were common in these subjects, with 62% of them reporting chronic musculoskeletal conditions, 21% reporting chronic endocrinological conditions (primarily diabetes), 17% reporting chronic respiratory conditions, and 16% reporting high-risk cardiovascular conditions such as previous myocardial infarction or cardiac surgery. The distribution of medical conditions was very similar in all 4 dose groups.

Vaccination was well tolerated in all groups; only 5 serious adverse events were reported ≤ 28 days after vaccination: 1 episode of chest pain (in a member of the TIV-dose group); 2 cases of pneumonia (1 each in the 15- μ g- and 45- μ g-dose groups); 1 case of gallstone-related pancreatitis (in the 15- μ g-dose group); and 1 case of *Staphylococcus aureus* bursitis in the elbow (in the 135- μ g-dose group). All of these episodes were judged by the investigator as unlikely to be related to the vaccination; as well, clinical laboratory studies on day 7 revealed no significant effects of vaccination in subjects enrolled in the first stage of the study.

The number of subjects who reported local or systemic side effects ≤ 7 days after vaccination are shown in table 1. Mild local pain was seen more frequently in the 135- μ g-dose group than in the TIV-dose group ($P = .012$); otherwise, the rates of local or systemic side effects were not statistically significantly different between the 4 dose groups. Moderate or severe complaints were reported in $<10\%$ of subjects, and there were no rate differences between the TIV-dose group and any of the 3 rHA0-dose groups.

Immune response. For 3 subjects—1 each in the TIV-, 15-

μ g-, and 45- μ g-dose groups—samples were unavailable, and HAI for the H1 component could not be performed in 1 subject in the TIV-dose group. Serological results for the remaining subjects are shown in table 2. In comparison with what was seen in the TIV-dose group, a $\geq 1:128$ titer against the H3 component of the vaccine occurred at a significantly higher rate ($P \leq .0001$) in the 45- μ g- and 135- μ g-dose groups and at a slightly higher rate ($P = .08$) in the 15- μ g-dose group. Therefore, the study's primary objective with respect to rHA0 was achieved in the 2 higher-rHA0-dose groups. Also, the rate of a ≥ 4 -fold-increased response was higher in the 45- μ g-dose group ($P = .003$) and the 135- μ g-dose group ($P < .001$) than in the TIV-dose group.

In addition, ANOVA controlling for baseline titer showed that the postvaccination GMT against the H3 component of the vaccine was higher in both the 45- μ g-dose group ($P = .0002$) and the 135- μ g-dose group ($P < .0001$) than in the TIV-dose group. A similar analysis of the H1-component- and the B-component-specific responses showed that the postvaccination GMT against both the H1 component ($P = .01$) and the B component ($P = .02$) was significantly lower in the 15- μ g-dose group than in the TIV-dose group; otherwise, there were no statistically significant differences between the 4 dose groups.

The rHA0 vaccine also induced NtA against the A/Panama/2007/99 (H3N2) virus in a dose-dependent manner; NtA responses as measured by microneutralization in a randomly chosen subset of subjects were significantly more frequent in recipients of high-dose rHA0 vaccine: ≥ 4 -fold-increased NtA responses were detected in 26.9% (7/26), 32.0% (8/25), 42.9% (12/28), and 70.4% (19/27) of the TIV-, 15- μ g-, 45- μ g-, and 135- μ g-dose groups, respectively ($P = .002$ for the TIV-dose group vs. the 135- μ g-dose group).

In contrast, the dose-dependent HAI response was not as pronounced for the H1 and B components of rHA0: both the rate of achieving a titer of $\geq 1:128$ and the rate of a ≥ 4 -fold-increased

Table 2. Immune response to vaccination.

Dose group	Subjects tested, no.	A/Panama/2007/99 (H3N2)				A/New Caledonia/20/99 (H1N1) ^a				B/Hong Kong/330/2001			
		GMT (95% CI)		Subjects, %		GMT (95% CI)		Subjects, %		GMT (95% CI)		Subjects, %	
		Before vaccination	After vaccination	Responding ^b	Titer $\geq 1:128$	Before vaccination	After vaccination	Responding ^b	Titer $\geq 1:128$	Before vaccination	After vaccination	Responding ^b	Titer $\geq 1:128$
TIV	98	42 (34–52)	103 (81–131)	33	49	15 (12–18)	38 (30–47)	37	21	53 (42–66)	132 (107–162)	34	63
rHA0													
15 μg	98	45 (34–58)	137 (103–183)	38	62	14 (12–18)	25 (19–33)	16	12	62 (47–83)	101 (76–135)	20	51
45 μg	99	43 (34–54)	216 (167–281)	55	76	18 (14–22)	42 (33–54)	32	26	65 (51–83)	114 (93–140)	24	65
135 μg	101	54 (43–67)	485 (352–667)	88	88	17 (14–22)	38 (29–49)	34	20	60 (47–77)	137 (111–170)	29	66

NOTE. CI, confidence interval; GMT, geometric mean titer; HAI, hemagglutination inhibition; rHA0, hemagglutinin expressed by recombinant virus in insect cells; TIV, trivalent inactivated influenza vaccine.

^a Responses to the H1 component were measured in only 97 subjects in the TIV-dose group.

^b Response was defined as a ≥ 4 -fold increase in HAI titer after vaccination, compared with that in serum before vaccination.

response were very similar in the 15- μ g-, 45- μ g-, and 135- μ g-dose groups. In both the 45- μ g- and 135- μ g-dose groups, both the rate of achieving a titer of $\geq 1:128$ and the response rates to the H1 and B components were similar to those in the TIV-dose group, whereas the 15- μ g-dose group had lower response rates than did the TIV-dose group ($P = .04$ for the B component; $P = .0007$ for the H1 component).

In the TIV-, 15- μ g-, 45- μ g-, and 135- μ g-dose groups, there were 24, 29, 27, and 28 subjects, respectively, who were ≥ 75 years old. Although these numbers are small, a subgroup analysis of these subjects showed no statistically significant intergroup differences in the response to the H1 and B components. Against the H3 component, however, the 135- μ g rHA0 vaccine was more immunogenic than was TIV, as it was in the 135- μ g-dose group as a whole: in these older subjects, the postvaccination GMTs (95% confidence interval [CI]) against the H3 component were 91 (61–135), 113 (72–179), 191 (121–302), and 349 (206–589), respectively, in the TIV-, 15- μ g-, 45- μ g-, and 135- μ g-dose groups ($P < .05$ for the 135- μ g-dose group vs. the TIV-dose group); the corresponding rates at which a postvaccination titer of $\geq 1:128$ was achieved were 38%, 54%, 81%, and 85% ($P < .001$ for either the 135- μ g- or the 45- μ g-dose group vs. the TIV-dose group).

DISCUSSION

The trivalent rHA0 vaccine evaluated in the present study was well tolerated even at doses as high as 135 μ g of each HA, or 405 μ g of total HA protein. There was a dose-response relationship for the H3 component, and both the 135- μ g and 45- μ g doses were more immunogenic than was the TIV dose. Antibody responses to the H1 and B components did not show the brisk dose-response relationship that was demonstrated for the H3 component, and the response rates were lower. However, both the levels of postvaccination antibody and the frequencies of responses to the H1 and B components in the 45- μ g- and 135- μ g-dose groups were similar to those seen in the TIV-dose group. These data suggest that rHA0 vaccine formulated at a dose of ~ 45 μ g per HA component, as determined by Lowry assay, or at a dose of ≥ 15 μ g per component, as determined by SRID, would have satisfactory performance in elderly adults and that, with respect to the H3 component, it might be superior to current TIV formulations.

The reasons why the present study did not find a similar response to the H1 and B components are not certain. In part, this result may reflect the lower actual doses of vaccine administered. In addition, electron microscopy used for morphological characterization of individual bulk-rHA0 preparations showed the presence of many more discrete spike-protein rosettes in the H3-component preparation than in either the B-component or the H1-component preparations, which had the least number of rosettes (data not shown). Further studies are needed to better understand the relationship between SRID-based results and pro-

tein content—and the role that these protein-rosette structures play in the immunogenicity of rHA0 vaccines.

Immunization with baculovirus-expressed HA induced functional antibody, as was reflected by both HAI and NtA responses. Although few data are available with regard to the protective efficacy of baculovirus-derived vaccine, a previous study has reported that baculovirus-derived monovalent H3-component HA vaccine appears to be protective against laboratory-confirmed influenza illness in healthy adults [2]. Thus, it is reasonable to expect that the HA vaccine used in the present study also would be protective in elderly adults. Field studies to document the protective efficacy of the trivalent, baculovirus-derived HA vaccine containing 45 μ g of each HA component, as determined by Lowry assay, or ≥ 15 μ g of each HA component, as determined by SRID, are currently under way.

Previous studies to assess the effect that increasing doses of influenza vaccine have on serum antibody response in elderly adults have generally shown that increasing doses are associated with higher levels of serum HAI and NtA [9–11], although, in studies evaluating multivalent preparations, dose-related increases in antibody against all of the components have not been seen [10, 12], a finding similar to the observations in the present study. Increasing doses of vaccine have also been associated with improved nasal antibody responses [11, 13] and with improved antibody-subclass response [14].

Although the potentially improved immune responses that might occur with the use of higher doses of influenza vaccine have been recognized for some time, production of higher-dose vaccines via current egg-based technology is problematic. Indeed, significant shortages and delays in the production of egg-grown vaccines have led to strategies to use reduced, rather than increased, doses of vaccine [15, 16]. A benefit of the efficient production of the HA antigen in insect cells by baculovirus-expression technology could include the feasibility of routine use of higher doses of vaccine. Future studies to evaluate the potential benefits of using this strategy in high-risk populations are needed.

Acknowledgments

We thank Diane O'Brien, Carolyn Nolan, Celsa Tajonera, Kate Knippa, Amy Hoepfer, Susan Parker and Vicki Smith, who assisted with the recruitment, vaccination, and follow-up of subjects in the trial, and Carol Smith, Heather Hill, and Thad Zajdow, who assisted with database management and statistical analysis. We also especially thank Rosalyn Battaglia, who performed all of the serological assays, and the NIAID Influenza Team: Sonnie Kim, Jean Hu-Primmer, Lydia Falk, and Linda Lambert.

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