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

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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.
and of the participating institutions were followed throughout the study.

Subjects were randomized to receive either trivalent subvi-

The study was conducted in 2 stages. In the first stage, 80

For 7 days after vaccination, subjects maintained a symptom

Vaccine. The HA genes of the 3 influenza viruses—A/Pana-

The working hypothesis for this trial was that a 20% im-

Immunogenicity analysis. Serum levels of antibody against
each of the 3 vaccine strains were measured independently of

Statistical methods. In the present study, the predefined

case of TIV, and modified Lowry assay of total protein con-

Comparisons of proportions were calculated by StatXact us-

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

<table>
<thead>
<tr>
<th>Subjects, no.</th>
<th>Experiencing symptoms after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose group</td>
<td>Local</td>
</tr>
<tr>
<td></td>
<td>Pain</td>
</tr>
<tr>
<td>TIV</td>
<td>99</td>
</tr>
<tr>
<td>rHA0 15 μg</td>
<td>99</td>
</tr>
<tr>
<td>rHA0 45 μg</td>
<td>100</td>
</tr>
<tr>
<td>rHA0 135 μg</td>
<td>101</td>
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</tbody>
</table>

**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 ≥37.5°C, moderate as ≥38.5°C, and severe as ≥39.5°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-μg- and 45-μg-dose groups); 1 case of gallstone-related pancreatitis (in the 15-μg-dose group); and 1 case of *Staphylococcus aureus* bursitis in the elbow (in the 135-μg-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 2. In comparison with what was seen in the TIV-dose group, a ≥1:128 titer against the H3 component of the vaccine occurred at a significantly higher rate (P = .001) in the 45-μg- and 135-μg-dose groups and at a slightly higher rate (P = .08) in the 15-μg-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-μg-dose group (P = .003) and the 135-μg-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-μg-dose group (P = .0002) and the 135-μg-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-μg-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-μg-, 45-μg-, and 135-μg-dose groups, respectively (P = .002 for the TIV-dose group vs. the 135-μg-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 ≥1:128 and the rate of a ≥4-fold-increased
<table>
<thead>
<tr>
<th>Dose group</th>
<th>Subjects tested, no.</th>
<th>A/Panama/2007/99 (H3N2)</th>
<th></th>
<th>A/New Caledonia/20/99 (H1N1)</th>
<th></th>
<th>B/Hong Kong/330/2001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before vaccination</td>
<td>After vaccination</td>
<td>Titer</td>
<td>Before vaccination</td>
<td>After vaccination</td>
<td>Titer</td>
</tr>
<tr>
<td>TIV</td>
<td>98</td>
<td>42 (34–52)</td>
<td>103 (81–131)</td>
<td>33</td>
<td>49</td>
<td>15 (12–18)</td>
</tr>
<tr>
<td>rHA0</td>
<td>15 μg</td>
<td>98</td>
<td>45 (34–58)</td>
<td>137 (103–183)</td>
<td>38</td>
<td>62</td>
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<tr>
<td></td>
<td>45 μg</td>
<td>99</td>
<td>43 (34–54)</td>
<td>216 (167–281)</td>
<td>55</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>135 μg</td>
<td>101</td>
<td>54 (43–67)</td>
<td>485 (352–667)</td>
<td>88</td>
<td>88</td>
</tr>
</tbody>
</table>

**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.

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

* 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-µg-, 45-µg-, and 135-µg-dose groups. In both the 45-µg- and 135-µg-dose groups, both the rate of achieving a titer of ≥1:128 and the response rates to the H1 and B components were similar to those in the TIV-dose group, whereas the 15-µg-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-µg-, 45-µg-, and 135-µg-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-µg rHA0 vaccine was more immunogenic than was TIV, as it was in the 135-µg-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-µg-, 45-µg-, and 135-µg-dose groups (P < .05 for the 135-µg-dose group vs. the TIV-dose group); the corresponding rates at which a postvaccination titer of ≥1:128 was achieved were 38%, 54%, 81%, and 85% (P < .001 for either the 135-µg- or the 45-µg-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 µg of each HA, or 405 µg of total HA protein. There was a dose-response relationship for the H3 component, and both the 135-µg and 45-µg 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-µg- and 135-µg-dose groups were similar to those seen in the TIV-dose group. These data suggest that rHA0 vaccine formulated at a dose of ~45 µg per HA component, as determined by Lowry assay, or at a dose of ≥15 µg 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 protein 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 µg of each HA component, as determined by Lowry assay, or ≥15 µg 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.

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References

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