Safety of High Doses of Influenza Vaccine and Effect on Antibody Responses in Elderly Persons

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**Background:** Immune responses after influenza immunization are reduced in elderly individuals, the group at greatest risk for complications and death after influenza. Improved vaccines are needed to address this problem.

**Methods:** Ambulatory individuals 65 years and older (N=202) were assigned randomly to receive a single intramuscular injection of the 2001-2002 formulation of trivalent inactivated influenza vaccine containing 15, 30, or 60 µg of hemagglutinin per strain (up to 180 µg total per dose) or placebo. Clinical and serologic responses were assessed during the month after immunization.

**Results:** Increasing dosages of vaccine elicited significantly higher serum antibody levels, frequencies of antibody responses, and putative protective titers after vaccination. Mean serum hemagglutination inhibition antibody titers 1 month after immunization in groups given 0-, 15-, 30-, and 60-µg dosages were 23, 37, 50, and 61 against influenza A/H1N1; 43, 86, 91, and 125 against influenza A/H3N2; and 10, 14, 18, and 24 against influenza B, respectively. Mean serum hemagglutination inhibition and neutralizing antibody levels against the 3 vaccine antigens in participants given the 60-µg dosage were 44% to 71% and 54% to 79%, respectively, higher than those in participants given the standard 15-µg dosage, and the 60-µg dosage level nearly doubled the frequency of antibody responses in those whose preimmunization antibody titers were in the lower half of the antibody range. Dose-related increases in the occurrence of injection site reactions were observed (P<.001), but all dosages were well tolerated.

**Conclusion:** The improved immunogenicity of high-dose influenza vaccine among elderly persons should lead to enhanced protection against naturally occurring influenza.

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INACTIVATED INFLUENZA VACCINES have been available in the United States for approximately 50 years for the prevention of influenza and its complications.\(^1\) They currently represent the primary means used to control influenza in developed countries, and their use is focused on preventing hospitalizations and death among persons at higher risk for these events, particularly the elderly.\(^2\)-\(^6\) However, for reasons unknown, vaccine efficacy among elderly persons has been variable.\(^7\)-\(^8\) A progressive reduction in immune competence is described with increasing age, and reduced antibody responses to inactivated influenza vaccines have been noted among elderly persons.\(^9\)-\(^11\) Because the level of serum antibody correlates with protection against influenza,\(^12\) investigation of ways to improve antibody responses to influenza vaccines among elderly persons is appropriate.

One approach to improve the immunogenicity of inactivated vaccines is to increase the dose of antigen contained in the vaccine. Previous studies\(^13\)-\(^24\) have shown that increasing the dose above the currently recommended level of 15 µg of the influenza virus hemagglutinin for each of the 3 virus strains in the vaccine results in dose-related increases in serum antibody responses. Although doses up to 405 µg of influenza hemagglutinin have been well tolerated and immunogenic, 60 µg per strain was selected as the highest dose to be tested based on current manufacturing considerations.

The purpose of this study is to assess the immunogenicity and reactogenicity of a current US vaccine formulation at increasing doses in the elderly population, which is considered to be in greatest need of an enhanced-potency vaccine.

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**METHODS**

**VACCINES**

Trivalent inactivated subvirion influenza vaccines (TIVs) containing strains matching the 2001-2002 formulation were manufactured by

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Aventis Pasteur, Swiftwater. Vaccines contained 15, 30, or 60 µg of each of the following influenza strains per 0.5-mL dose: A/New Caledonia/20/99 (H3N2), A/Panama/2007/99 (H3N2), and B/Victoria/504/2000. The placebo was sterile isotonic sodium chloride solution.

**CLINICAL PROCEDURES**

Ambulatory, medically stable persons at least 65 years old were recruited from the Texas Medical Center (Houston) and from local volunteer groups and senior citizens’ organizations. Written informed consent was obtained from each participant in accordance with protocols approved by the Baylor College of Medicine institutional review board. Participants were stratified according to receipt of TIV during the preceding influenza season and then were randomized to receive a single intramuscular dose of placebo or TIV containing 15, 30, or 60 µg of hemagglutinin per strain. Oral temperature, injection site, and systemic symptoms and signs were recorded in a diary daily for 1 week after immunization. Participants were examined 30 minutes and 2 and 28 days after inoculation and were contacted 6 months after immunization to assess for the occurrence of serious adverse events (AEs) since vaccination. Blood samples for antibody assays were obtained before and 1 month after immunization. All the participants were offered the most up-to-date commercial vaccine before the start of the influenza season.

**DEFINITION OF VACCINE REACTIONS**

Injection site and systemic symptoms (headache, malaise, nausea, and body ache) were graded on a scale from 0 to 3 (0 = absence of the symptom; 1 = mild, easily tolerated; 2 = moderate, interferes with activity; and 3 = severe, incapacitating). An oral temperature of 37.6°C or greater was considered a fever. The presence of the symptom; 1 = mild, easily tolerated; 2 = moderate, interferes with activity; and 3 = severe, incapacitating). An oral temperature of 37.6°C or greater was considered a fever. The presence of the symptom; 1 = mild, easily tolerated; 2 = moderate, interferes with activity; and 3 = severe, incapacitating). An oral temperature of 37.6°C or greater was considered a fever. The presence of the symptom; 1 = mild, easily tolerated; 2 = moderate, interferes with activity; and 3 = severe, incapacitating). An oral temperature of 37.6°C or greater was considered a fever. The

**LABORATORY PROCEDURES**

Serum hemagglutination inhibition (HAI) and neutralizing antibody assays were performed as described previously. For the HAI antibody test, concentrations of reagents were altered, and an erythrocyte adsorption step was added to permit a starting dilution of 1:4. Antigens for HAI assays were whole virus A/Panama/2007/99 (H3N2), A/New Caledonia/20/99 (H1N1), and B/Victoria/504/00. The same viruses were used in neutralizing tests for influenza B and influenza A/H1N1 antibody, but A/Moscow/10/99 (H3N2), antigenically similar to A/Panama/H3N2 virus, was used in influenza A/H1N1 tests. A 4-fold or greater increase in HAI or neutralizing titers from baseline to 1 month after immunization was considered significant. For protective antibody titers, an HAI titer of 32 or greater was used as a level likely to convey protection. Putative protective titers for neutralizing tests have not been defined.

**STATISTICAL CONSIDERATIONS**

The primary reactogenicity end points were the frequency and severity of solicited local and systemic AEs. The primary immunogenicity end point was the geometric mean titer of serum HAI and neutralizing antibodies against each of the 3 vaccine antigens 1 month after immunization. Secondary immunogenicity end points included the proportion of participants in each group who achieved an HAI titer of at least 32 after vaccination and the rates of 4-fold or greater increases in titer after immunization. Exploratory analyses for factors that affect reactions and immune responses included sex, age, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), previous vaccination, and report of respiratory illness during the preceding season.

We hypothesized that increasing the dose of TIV from 15 to 60 µg of hemagglutinin per strain would be well tolerated and would elicit significantly higher geometric mean titers of serum antibody 1 month after immunization. A sample size of 50 individuals per group had 80% power to detect a 75% increase in antibody titer for at least 1 antigen at the P = .05 significance level, based on estimates derived from previous studies.

Differences in proportions were compared using the Fisher exact test, and continuous variables were compared using analysis of variance. The dose-response relationships for the frequencies of antibody responses were analyzed using the Cochran-Armitage test for trend. Logistic regression models were used to assess whether dichotomous clinical and serologic responses were affected by vaccine dose, age, sex, BMI, previous immunization, premunition titers, and report of influenza-like illness during the preceding winter. Linear least squares regression analysis was used to assess the dose response of the antibody titers, controlling for covariates as in the logistic regression models. Age and BMI were entered into the regression models as continuous variables, whereas sex, previous vaccination, and report of previous respiratory illness were included as dichotomous variables. We also tested the effect of age as a dichotomous variable (< 75 vs ≥ 75 years). Because we were interested in the impact of each of these variables on the outcome, no selection procedures were used to retain only significant variables in the model. We tested for interactions of age with BMI, sex with BMI, age with previous influenza vaccination, and sex with previous influenza vaccination. In general, these interactions were not significant, and, as a result, we report the main-effects models only.

**RESULTS**

Two hundred two individuals were enrolled between June 18, 2002, and October 4, 2002. The median age of the participants was 72.5 years (age range, 65-88 years; mean age, 72.4 years). The mean age of women (71.9 years) was similar to that of men (72.8 years). All the participants completed follow-up. There were no significant differences among the vaccine groups regarding baseline demographic characteristics, antibody titers against vaccine antigens, or the percentage of individuals with a preimmunization titer of at least 32. Forty-one percent of the participants were women, and 97.5% were white. Eighty-two percent of the participants reported receipt of TIV the preceding influenza season, and 18% reported an influenza-like illness during the previous winter. Previously vaccinated participants had higher antibody titers against the influenza A/H1N1 and influenza B antigens before immunization compared with those who had not been vaccinated the preceding season (P < .001 for influenza A/H1N1 HAI and neutralizing antibody titers; P < .001 and P = .02 for influenza B HAI and neutralizing antibody titers, respectively; analysis of variance) and were also more likely to have a preimmunization HAI titer of at least 32 against all vaccine antigens except in-
Antibody Responses

Serum antibody responses are summarized in Table 1, Figure 2, and Figure 3. Significant differences in geometric mean titers 1 month after immunization were observed among groups for all test antigens (H1N1, H3N2, and influenza B) and test types (HAI and neutralizing) (P<.001 for all; analysis of variance) (Table 2). In all cases, there were dose-related increases in geometric mean titers and in the percentage of participants with a putative protective titer (HAI titer ≥32) (Figure 2). Dose-related increases in the frequency of significant antibody responses were observed for all antigens except influenza B in the HAI test (Figure 3). The dose-related increases in neutralizing antibody response frequencies were greatest for participants whose preimmunization antibody titers were in the lower half of the antibody range (Table 3). Among these participants, responses to the 60-µg dose vs the 15-µg dose to each of the 3 antigens in those with preimmunization neutralizing titers were nearly doubled. Similar trends were observed for HAI antibody responses (data not shown). Almost all the participants with very low titers responded to the 60-µg dose compared with poor responses to the 15-µg dose. The frequency of 4-fold or greater increases in titers among individuals with preimmunization HAI titers of 8 or less against influenza A/H1N1 were 60% (6/10), 82% (9/11), and 100% (5/5) for the 15-, 30-, and 60-µg dose groups, respectively. For influenza A/H3N2, these frequencies were 50% (4/8), 63% (5/8), and 80% (4/5), respectively.

In linear regression models, there was a significant dose effect when controlling for age, sex, BMI, previous immunization, previous influenzalike illness, and preimmunization titer (Table 3). The variable estimates for the dose levels in Table 3 indicate the increase in the serum antibody level associated with a higher vs lower dose. For example, if all other independent variables were the same, an individual with a dose of 15 µg would have an HAI level for influenza A/H1N1 that is 0.77 log 2 units higher than that of an individual who received placebo. Female sex was associated with higher antibody titers for all comparisons (P<.05 for all) except influenza B in the HAI test and influenza A/H1N1 in the neutralizing test. Higher titers before immunization were associated with higher titers after immunization for all comparisons (P<.001 for all). Postimmunization serum antibody titers among participants who were vaccinated during the previous season were significantly lower than among those who were not vaccinated during the previous season for all antigens and test types (P<.01) except influenza A/H1N1 in the HAI test when controlling for age, sex, BMI, and pretiters; similar trends were observed for the proportion of individuals with a 4-fold increase in titer and for those achieving an HAI titer of 32 after immunization (data not shown).

Participants who had been immunized the previous influenza season did not differ significantly from those who had not been immunized regarding mean age, BMI, sex distribution, and proportion reporting a previous influenzalike illness (data not shown). Previously unvaccinated participants were somewhat more likely to re-

Figure 1. Percentage of participants reporting injection site reactions during the week after immunization for each vaccine dose group. Dark bars=moderate (discomfort) or small (redness/swelling); light bars=mild (discomfort) or medium or large (redness/swelling). See the “Methods” section for details.
influenza vaccines elicit significant responses in most susceptible younger adults, their immunogenicity is lower among elderly and other persons who are at high risk for complications and death. Improved vaccines are needed to reduce the morbidity and mortality associated with influenza in these vulnerable populations. Increasing the dose of inactivated influenza vaccine consistently and safely increases the immunogenicity of inactivated influenza virus vaccines; moreover, vaccines that contain higher doses have conferred significantly enhanced protection against naturally occurring influenza. Increased dose has also been shown to increase (1) antibody responses to heterotypic strains in humans (W.A.K. et al, unpublished data, 2004) and (2) protection against heterotypic strains in an animal model. High-dose vaccines also may overcome the apparent age-related decrements in cross-reactivity of antibodies induced by vaccination, as observed by de Jong et al. These responses are particularly important in circumstances of antigenic mismatch between vaccine and epidemic strains. Antigenic mismatch between vaccine and circulating influenza A/H3N2 viruses, the subtype responsible for the highest morbidity and mortality rates among elders, has occurred on several occasions in recent years. Although other researchers have successfully conducted studies using higher doses of inactivated influenza vaccines, the current dose-response study was clearly needed for several reasons. This study focused on the current population of elderly individuals, a target group with the greatest need for more efficacious influenza vaccines. In addition, since the previous studies were conducted, manufacturing of inactivated influenza vaccines has improved greatly, quantification of the influenza antigen content of vaccines has become more exact, and the current influenza A/H1N1, influenza A/H3N2, and influenza B strains have undergone antigenic drift for many years. It is important to carefully test for safety and to determine the dose for optimal immunogenicity in this target group before larger-scale studies are considered.

This study confirms the safety and improved immunogenicity of a contemporary vaccine based on a licensed product in a dose range considered feasible for production. Although the higher doses of vaccine elicited higher rates of injection site reactions, most of these reactions were mild and transient. As observed previ-

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<th>Table 1. Geometric Mean Serum HAI and Neutralizing Antibody Responses Before and 1 Month After Immunization*</th>
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<td><strong>Antibody Titer, Geometric Mean (95% CI)</strong></td>
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<td><strong>Influenza A/H1N1</strong></td>
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Abbreviations: CI, confidence interval; HAI, hemagglutination inhibition.
*We tested for differences among the doses in the geometric mean titers before immunization using analysis of variance. None of these differences were statistically significant. Differences among doses after immunization were analyzed using linear regression models (Table 3).

**Figure 2.** Percentage of participants with a serum hemagglutination inhibition antibody titer of 32 or greater after immunization for each vaccine dose group.

Annual epidemics of influenza are regularly associated with excess mortality, and most of these deaths occur among persons older than 65 years. Immunization of ambulatory and institutionalized elderly individuals with inactivated influenza vaccines has been associated with significant reductions in hospitalization and death in cohort studies, but their efficacy against infection and illness has been variable. Despite reductions noted in cohort studies, the number of influenza-associated hospitalizations and excess deaths associated with influenza have continued to rise in recent years despite increasing vaccine coverage.

Although currently recommended doses of inactivated influenza vaccines elicit significant responses in most susceptible younger adults, their immunogenicity is lower than that of higher doses among elderly and other persons who are at high risk for complications and death. Improved vaccines are needed to reduce the morbidity and mortality associated with influenza in these vulnerable populations. Increasing the dose of inactivated influenza vaccine consistently and safely increases the immunogenicity of inactivated influenza virus vaccines; moreover, vaccines that contain higher doses have conferred significantly enhanced protection against naturally occurring influenza. Increased dose has also been shown to increase (1) antibody responses to heterotypic strains in humans (W.A.K. et al, unpublished data, 2004) and (2) protection against heterotypic strains in an animal model. High-dose vaccines also may overcome the apparent age-related decrements in cross-reactivity of antibodies induced by vaccination, as observed by de Jong et al. These responses are particularly important in circumstances of antigenic mismatch between vaccine and epidemic strains. Antigenic mismatch between vaccine and circulating influenza A/H3N2 viruses, the subtype responsible for the highest morbidity and mortality rates among elders, has occurred on several occasions in recent years. Although other researchers have successfully conducted studies using higher doses of inactivated influenza vaccines, the current dose-response study was clearly needed for several reasons. This study focused on the current population of elderly individuals, a target group with the greatest need for more efficacious influenza vaccines. In addition, since the previous studies were conducted, manufacturing of inactivated influenza vaccines has improved greatly, quantification of the influenza antigen content of vaccines has become more exact, and the current influenza A/H1N1, influenza A/H3N2, and influenza B strains have undergone antigenic drift for many years. It is important to carefully test for safety and to determine the dose for optimal immunogenicity in this target group before larger-scale studies are considered.

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ously, female sex was associated with higher levels of injection site discomfort in multivariate analyses, but all the doses were well tolerated. Lower BMI also was associated with increased injection site reactogenicity. No serious adverse reactions or increases in the rates of systemic reactions were observed.

In multivariate analyses, the dose was the most important factor associated with an improved antibody response.
sponse. Several other variables were shown to relate to antibody responses. Female sex was associated with an improved antibody response. Sex-related differences in immune responses (immunoglobulin levels, autoimmune diseases, etc) previously have been reported; these have been attributed to hormonal effects on various components of the immune system. These effects decline in postmenopausal women, but they may be enhanced by hormone therapy. We did not relate the improved immunogenicity in women to hormone therapy, although the sample size is small. Participants who received influenza vaccine the preceding influenza season had significantly lower frequencies of antibody responses and lower serum antibody levels after immunization compared with participants who did not receive vaccine the preceding season. Previously vaccinated participants had a somewhat higher frequency of underlying medical conditions, possibly accounting for some of these differences. Repeated annual immunization of healthy younger adults has been associated with somewhat lower antibody responses against influenza A/H3N2 and influenza B antigens in a randomized clinical trial. However, note that improved immunogenicity of vaccines containing higher antigen content was seen in previously immunized individuals despite the fact that they received a vaccine containing the same antigens less than a year earlier.

Several limitations of this study will need to be addressed in future, expanded studies. Only healthy ambulatory elderly individuals were recruited, and responses in other, less vigorous elderly persons may differ. Although the safety and reactogenicity profiles were favorable, the sample size was modest. Participants received influenza vaccine formulations containing the same antigens present in the previous year’s vaccine, and they were immunized less than 1 year after receipt of their most recent influenza vaccine. Because the study was conducted in a single season, the safety and immunogenicity of repeated vaccination with high-dose vaccines is not known. The effectiveness of immunization with enhanced-potency vaccines will need to be assessed among large groups of elderly individuals who are immunized in a timely manner with the most up-to-date formulations.

The results of this study show that the highest dose (60 µg) was the most efficacious. Higher doses were not tested because of manufacturing considerations. The increased doses tested in this study were selected because they would not significantly affect projected future vaccine supply. Although adjuvant vaccine could potentially reduce the antigenic dose, adjuvant vaccines used for annual vaccination in elderly individuals present unknown risks compared with the safety record of nonadjuvant vaccines. Our findings provide a rationale for further, expanded trials designed to assess the effectiveness of immunization with enhanced-potency vaccines in the elderly population.

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