Comparison of Immunogenicity of a Whole Virion and a Subunit Influenza Vaccine in Adults

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The immunogenicity and reactogenicity of a whole virus (Merck Sharp & Dohme) and a subunit (Wyeth) influenza A/New Jersey/76 vaccine were compared in a group of 214 normal adult subjects. Both the seroconversion rate and the magnitude of hemagglutination inhibition antibody response were significantly ($P < 0.01$) lower in the recipients of the subunit vaccine, whereas there were no significant differences in local or systemic reactions between the two preparations. On the basis of these data, we question the previous Public Health Service recommendation that one dose of either preparation of the influenza A/New Jersey/76 vaccine is equally efficacious in individuals over 24 years of age.

Early influenza vaccine preparations, when administered in immunogenic doses, were generally associated with unpleasant side reactions, which were often as uncomfortable and debilitating as the symptoms of naturally acquired influenza. Accordingly, efforts were directed toward improving the quality of influenza vaccines. The introduction of large-scale zonal ultracentrifugation to purify crude egg-grown virus resulted in the production of vaccine preparations of high purity (3, 11). Such vaccines, when given in doses which contain a sufficient mass of antigen to be highly immunogenic, are virtually free of serious side effects.

The development of disrupted virus vaccines, by controlled degradation of virions into hemagglutinin and neuraminidase subunits, represents a further refinement of vaccine quality. Davenport et al. (4) in 1964 demonstrated that immunization of humans with subunit influenza vaccine led to the development of antibody levels equivalent to those seen in subjects who received comparable amounts of whole-virus vaccine (WVV). Subsequent studies on the efficacy of subunit vaccines in mice have shown that these vaccines are poor immunogens; however, the data in humans have been conflicting (1, 2, 8). Nonetheless, since 1969, subunit vaccines have been commercially available for immunization of humans. On the basis of information derived from the National Influenza Immunization Program (10), the influenza A/New Jersey/76 WVV and split-product vaccine (SPV) were shown to be equally immunogenic in individuals over 24 years of age. Accordingly, it was recommended that one dose of 200 chick cell-agglutinating

(CCA) units of either WVV or SPV would be appropriate and sufficient for immunization of individuals in this age group.

An influenza immunization program at this institution, in which individuals received either a WVV or a SPV according to the Public Health Service recommendation, was completed at the end of 1976. Hemagglutination inhibition (HAI) antibody levels measured in a representative group of participants indicated a striking difference in the responses between recipients of the WVV and those of the SPV. Consequently, HAI antibody titers were determined for the entire study population to explore in greater detail the comparative responses in humans to influenza A/New Jersey/76 WVV and SPV. The results of this study are herein reported.

MATERIALS AND METHODS

Study population. Employees of The Jewish Hospital of St. Louis comprised the study population. The subjects were randomly allocated to receive either the WVV or the SPV. The individuals were questioned at the time of immunization regarding the presence of any known cardiovascular, renal, or bronchopulmonary disease and were excluded from the study if any of these underlying conditions were present. Voluntary informed consent was obtained from all of the subjects before immunization. All individuals with an allergy to eggs or egg products were excluded.

Serum collection. Blood samples were obtained before immunization and again at an interval of 3 to 4 weeks postimmunization, and serum was stored at -20°C .

Influenza vaccines. All subjects received an intramuscular injection of 0.5 ml of either WVV or SPV, containing 200 CCA units of type A/New Jersey/76. The WVV used was manufactured by Merck Sharp

& Dohme (West Point, Pa.), and the SPV used was produced by Wyeth Laboratories (Philadelphia, Pa.). The vaccines were obtained from the St. Louis City Health Department and stored at 4°C from the time they were received until the time of administration. Precise potency testing was not performed in this laboratory.

Vaccine reactions. All subjects were instructed to report the presence of erythema, induration, and tenderness at the injection site during the first 48 h postimmunization. Constitutional signs and symptoms including fever, malaise, chills, headache, nausea, vomiting, and any other complaints were also recorded. Reactions other than fever were graded as absent, slight, moderate, and severe, and an oral temperature over 38°C (100°F) within the first 48 h after immunization was considered to be a febrile response. The subjects were relied upon to take their own temperature, and examinations were performed by one of us in symptomatic individuals. A placebo group was not evaluated.

Antibody measurements. Serum antibody titers against the influenza A/New Jersey/76 antigen were measured by the HAI microtiter method (5). Specimens were treated with trypsin and potassium periodate to remove nonspecific inhibitors of hemagglutination. Fresh chicken erythrocytes were used, and reference sera of known identity and titer were included as controls in each test. Serial twofold dilutions of the sera were carried out from 1:10 to 1:640. A patient's pre- and postimmunization specimens were always assayed for HAI antibody in the same test, and equal numbers of patients' sera from both vaccine groups were assayed simultaneously.

Statistical methods. The chi-square test with the Yates correction for continuity and the *t* test for independent samples were used in the comparison of proportions and means. Logarithmic transformation was applied to the antibody titers and ratios. For geometric mean titer calculations, sera which demonstrated no antibody at the lowest dilution (1:10) were considered as positive at 1:5.

RESULTS

Characteristics of recipient groups. Of the 214 individuals who participated in the study, 118 (55%) received the SPV, whereas 96 (45%) were immunized with the WVV. The sex and racial composition of the two groups of recipients was nearly identical. The mean age of the SPV subjects was lower than that of the WVV subjects (33.6 years versus 37.1 years), but the dif-

ference was not significant. Eighty-one percent of the subjects in each recipient group were between the ages of 23 and 52 years. However, the number of individuals in the 17- to 23-year-old age bracket who received the SPV was significantly higher than the number receiving the WVV (20 versus 3, $P < 0.05$), whereas the converse was true in the ≥ 52 -year-old age bracket (3 versus 15, $P < 0.05$).

Seroconversion. Ninety-four percent of the subjects immunized with the WVV responded with a fourfold or greater increase in HAI antibody titer (Table 1), which is significantly higher than the 61% response rate seen in those individuals who received the SPV ($P \ll 0.001$). The magnitude of the antibody response, as measured by both the absolute postimmunization geometric mean titers and the ratio of post- to preimmunization titers, was also significantly greater in the WVV group ($P \ll 0.001$).

When the seroconversion rates and the HAI antibody titers were examined according to age, within each vaccine group, no significant differences were seen in responses between the various age groups. However, when the WVV and SPV recipient groups were compared according to age (Figure 1), the response rate was shown to be significantly higher in the WVV subjects aged 24 to 34 years and 35 to 51 years compared with subjects of the same age who received the SPV ($P \ll 0.001$). This relationship was also evident in these same age groups with respect to the magnitude of the antibody response ($P \ll 0.001$). The uneven distribution of patients in the 17- to 23-year and ≥ 52 -year age brackets did not permit statistical comparisons.

There was no significant difference between response rates as related to the presence or absence of preexisting A/New Jersey/76 antibodies before immunization. However, only three of the subjects who received the SPV had preimmunization antibodies, which is significantly less than the 25 individuals with preexisting antibody who received the WVV ($P < 0.05$).

Vaccine reactions. Slight local reactions at the site of injection were reported by eight individuals receiving the WVV and by five SPV recipients. Systemic reactions were distinctly

TABLE 1. Seroconversion^a rates and geometric mean titers

Vaccine	No. tested	No. with seroconversion ^a (%)	GMT ^b		
			Preimmunization	Postimmunization	Ratio of post- to preimmunization
Whole virus	96	90 (94)	6.6	109.1	16.5:1
Split virus	118	72 (61)	5.1	38.6	7.5:1

^a Fourfold or greater increase in HAI antibody titers postimmunization.

^b GMT, Geometric mean titer. For geometric mean calculations, a titer of $<1:10$ was classified as 1:5.

FIG. 1. Age-specific rates of seroconversion to influenza A/New Jersey/76 vaccine. Symbols: (■) WVV recipients; (□) SPV recipients. Number in parentheses is total number of subjects tested in each age group.

uncommon with either vaccine and, when present, tended to be slight and transient. A temperature of $>38^{\circ}\text{C}$ (100°F) was noted by 5 and 2.6% of the WVV and SPV recipients, respectively, but in no subjects did the febrile response persist beyond 48 h. There was no significant difference in reactogenicity between the two vaccine preparations. No allergic reactions were observed in any of the participants.

DISCUSSION

In several trials influenza A virus subunit vaccines have been shown to be as immunogenic as the zonally purified whole-virus conventional vaccine preparations in both children and adults, while being almost devoid of reactogenicity (6, 8, 12). Other data (2, 14) derived from studies in both experimental animals and humans, however, have shown the SPVs to be significantly less immunogenic than the WVVs, particularly in subjects without serological evidence of previous exposure to similar influenza viruses. A detailed report from the Food and Drug Administration (1) also documented the decreased antigenicity of the SPVs in immunologically inexperienced volunteers, although the vaccines were all administered subcutaneously despite the recommended intramuscular route for SPVs.

The present data clearly demonstrate the immunogenic inferiority of the A/New Jersey/76 SPV (Wyeth) in normal adult subjects. The WVV preparation used in this study was highly effective in stimulating antibody production in individuals with and without preimmunization antibody titers. The number of subjects in the SPV group who had preimmunization antibodies to the A/New Jersey/76 antigen was too small to evaluate, but nonetheless only 60% of the individuals without antibodies exhibited seroconversion. These results, though generally con-

sistent with the pooled data from the Influenza Program of the National Institute of Allergy and Infectious Diseases (10), are different in that the overall rate of seroconversion in response to 200 CCA units of WVV (Merck Sharp & Dohme) was higher in our series (94 versus 78%), as were the seroconversion rates to 200 CCA units of the SPV (Wyeth Laboratories) (61 versus 50%).

The basis for the apparent inferior immunogenicity of the SPVs demonstrated in this and other studies remains speculative. It has been postulated (1) that the size of the split-product antigens might be too small to be handled optimally by the immune system. It has also been shown with inactivated measles vaccine that, although the hemagglutinin titer increases markedly after splitting the whole-virus preparations into subunits, such disruption obviously does not alter the total amount of viral antigen (9). Consequently, 200 CCA units of whole virus actually represents a much larger antigenic mass than 200 CCA units of split virus. All things considered, it seems most likely that the immunogenic inferiority of the SPV product used in this study is simply due to its lower antigen content rather than to any inherent practical or theoretical limitation of subunit vaccines in general. Whatever the basis for the observed differences, the WVV (Merck Sharp & Dohme) and the SPV (Wyeth Laboratories) were clearly not equivalent immunogens.

Since clinical protection after immunization is directly related to the level of serum antibody (7), the significantly greater magnitude of antibody response induced by the WVV would recommend its use to achieve optimal clinical effectiveness. It thus appears that the advantages derived from the relatively minor reduction in the frequency of vaccine reactions afforded by the influenza A/New Jersey SPV (Wyeth) are essentially negated by the immunogenic inferiority of this preparation at recommended dosage levels. We therefore question the 1976 Public Health Service Advisory Committee on Immunization Practices, which, having regarded all licensed influenza A/New Jersey vaccine preparations as equivalent to one another regardless of antigen content, recommended that one dose of either influenza A/New Jersey WVV or SPV will suffice in persons over 24 years of age (13). Whereas the present data obviously cannot be directly extended to the influenza vaccines in current use, nonetheless it seems clear that more meaningful characterization of vaccine immunogenicity or antigen content is needed to aid the practicing physician in the selection of the vaccine preparation most likely to benefit his (her) patients.

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