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# Evaluation of a Live, Attenuated Recombinant Influenza Vaccine in High School Children

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A live, attenuated, recombinant influenza vaccine (Alice strain) administered intranasally was evaluated in high school students and compared with intranasal placebo and subcutaneous, inactivated, bivalent influenza vaccine. The Alice strain was antigenic, increasing the geometric mean titer (GMT) from a prestudy level of 30.2 to a postvaccine level of 189.6. The inactivated vaccine increased the GMT from 32.9 to 361.8. There was no increase in the GMT among the placebo recipients. The Alice strain produced little reaction. With an antigenic, safe, acceptable, live, attenuated influenza vaccine available, immunization on a widespread basis should be considered.

Influenza epidemics are commonplace and are the cause of significant excessive mortality. The best available method to protect against influenza is through immunization. Currently available influenza vaccines are inactivated preparations that have been shown to have variable effect in protecting against influenza and to stimulate antibody of relatively short duration, and they frequently are comprised of outdated antigens because of major abrupt antigenic changes by the circulating influenza viral strains (3, 13). To affect these shortcomings, live, attenuated influenza vaccines and recombinant influenza vaccines have been developed and are being evaluated (1, 5, 7). In addition, current vaccine utilization ignores massive, widespread immunization and concentrates on selected high-risk groups (9). Although children are thought to be important vectors in the propagation of community influenza outbreaks, immunization of children has not been recommended.

The purpose of this study was to evaluate the reactivity and immunogenicity of a live, attenuated recombinant influenza A vaccine in children and to compare it with inactivated influenza vaccine and placebo.

## MATERIALS AND METHODS

**Study subjects.** The study subjects were the students in grades 9 through 12 at the Wyoming High School, Wyoming, Ohio. Signed, informed consent was obtained from the parents and the subjects. The study had the approval of the University Committee on Research.

**Medications.** The origin of the live, attenuated influenza vaccine, Alice strain (kindly supplied by Smith, Kline & French Co., Philadelphia, Pa.), was the recombination by G. C. Schild, W.H.O. Influenza Centre, Mill Hill, London, of the third embryonated egg passage of the A/England/42/72 strain with the Mount Sinai A/PR8/34 strain, which had been passed through a human volunteer and reinoculated in specific-pathogen-free (SPF) eggs. Allantoic fluid was harvested after 24 h of incubation and treated with anti-A/PR8/34 serum to neutralize residual virus. The purpose of this recombination was to obtain a strain with high growth efficiency. Viral clones producing high virus yields were produced after four terminal dilution passages in SPF eggs. This recombinant, called MRC-2, was shown to have the hemagglutinin and the neuraminidase of the A/England/42/72 subtype. The MRC-2 clone virus was received after its fourth terminal dilution passage and was passed in SPF eggs once more. Next, it was passed twice in eggs in the presence of heated guinea pig serum to render it inhibitor resistant. Three further passages at terminal dilutions were carried out in the absence of serum to clone the obtained resistant mutant and check the stability of the resistant marker. Virus harvested at each passage was completely resistant against normal horse and guinea pig serum inhibitors. The virus passage at this level was used as inoculum for the Alice strain seed lot.

The experimental lot, 2F-1211, used for the study was the 12th passage of the MRC-2 clone and the fifth passage in the absence of serum inhibitors. The dose used was $10^{7.6}$ mean egg infectious doses, contained in 10 drops given intranasally, 5 drops per nostril. The vaccine was provided as a vial of lyophilized material containing one dose. It was reconstituted with 5% sucrose.

One-half milliliter of inactivated influenza virus bivalent vaccine (Fluogen) was given subcutaneously, containing 700 chick cell agglutination units of A/

England/42/72 and 300 chick cell agglutination units of B/Mass/1/71 antigens. This was commercially available vaccine.

**Placebo.** Placebo (kindly supplied by Smith, Kline & French Co.) was prepared in a similar manner as the Alice strain except that no virus was inoculated. It was lyophilized and reconstituted in the same manner as the Alice strain.

**Study plan.** Participating students were interviewed at school. Ten milliliters of venous blood was collected and then one of three medications was administered: 10 drops of Alice strain intranasally; 10 drops of placebo intranasally; or 0.5 ml of inactivated bivalent influenza vaccine subcutaneously. Immediate reactions to medications were noted. For the next 6 days, students were interviewed daily, signs and symptoms were scored (0 = not present; 1 = mild; 2 = moderate; 3 = severe), and oral temperatures were recorded. On day 7, students who had received the intranasal medications received a second dose. Signs, symptoms, and temperatures were recorded for the next 6 days. A second blood specimen was collected on day 30 from all students.

**Follow-up evaluation.** Weekly interviews of the students were conducted for the next 14 weeks or through the duration of the influenza season. Signs and symptoms were scored as before. In the event a student developed an influenza-like illness, attempts were made to examine the student during the illness, and specimens for viral culture and serological tests were collected. In addition, a third blood specimen for serological assay was collected at the end of the follow-up period from students who had a history of influenza-like illness during the study period.

**Laboratory studies: serologic assays.** The sera were tested for hemagglutination inhibition antibodies by the usual technique (12). Antigens for the hemagglutination inhibition assays included the Alice, B/Mass, and B/Hong Kong strains. Sera were always paired and tested simultaneously.

**Virus isolation.** Influenza viruses were isolated and identified in rhesus monkey kidney tissue culture cells by the hemadsorption test system (9). Specific hyperimmune antisera were kindly obtained from the Center for Disease Control.

## RESULTS

One hundred and twenty-six students participated in the study. Seventy-four students received Alice strain vaccine, 28 students received intranasal placebo, and 24 students received inactivated bivalent commercial influenza vaccine subcutaneously. The ages of the students varied from 13 to 18 years. Forty-three girls and 31 boys received the Alice strain vaccine; 12 boys and girls and 14 boys and girls received inactivated vaccine and placebo, respectively.

The serological results are summarized in Table 1. Among the Alice strain vaccines, 62.2% of the students developed a significant rise

TABLE 1. *Influenza vaccine serological results*

| Group | Prevaccination titer | No. with postvaccination titer of: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <10 | 10 | 20 | 40 | 80 | 160 | 320 | 640 | 1,280 | 2,560 |
| Alice | <10 | | | | 1 | 1 | 1 | 2 | 2 | 1 | 1 |
| | 10 | | | | 1 | 1 | 1 | | 1 | | |
| | 20 | | | | 2 | 3 | 4 | 2 | 2 | | |
| | 40 | | | | 3 | 3 | 3 | 5 | 4 | 2 | |
| | 80 | | | | | 5 | 4 | 2 | 2 | | 1 |
| | 160 | | | | | | 4 | 2 | 1 | | |
| | 320 | | | | | | | 3 | 1 | 1 | |
| | 640 | | | | | | | | | 1 | 1 |
| Inactivated vaccine | <10 | | | | | | 1 | | | | |
| | 10 | | | | | | | | | | |
| | 20 | | | | | | 1 | 3 | 3 | 1 | |
| | 40 | | | | | 2 | | | 3 | | 2 |
| | 80 | | | | | 1 | 1 | 1 | 1 | | 1 |
| | 160 | | | | | | | | 2 | | |
| | 320 | | | | | | | | | | |
| | 640 | | | | | | | | 1 | | |
| Placebo | <10 | 1 | | | | | | | | | |
| | 10 | | | 1 | | | | | | | |
| | 20 | | | 4 | 1 | | | | | | |
| | 40 | | | | 9 | 2 | | | | | |
| | 80 | | | | | 3 | 1 | | | | |
| | 160 | | | | | 1 | 3 | | | | |
| | 320 | | | | | | | 2 | | | |
| | 640 | | | | | | | | | | |

(fourfold or greater) in antibody titer. The geometric mean titer (GMT) increased from a prevaccine level of 30.2 to a postvaccine level of 189.6, or a sixfold rise. There were nine Alice strain vaccinees who lacked preexisting antibody to Alice strain. All nine developed significant antibody levels with a GMT of 276.2. Twenty-six Alice strain vaccines had a preexisting antibody titer of 20 or less. Twenty-four (92.3%) of these students developed a significant rise in antibody titer with a GMT of 158.5. One student with preexisting antibody titer of 640 experienced a fourfold rise. There was no significant difference in seroconversion rates in GMT achieved between the boys and girls or between the various age groups.

Among the inactivated influenza bivalent vaccines, 19 of 24 (79.2%) developed a significant increase in antibody titer, with a prevaccine GMT of 32.9 and a postvaccine GMT of 361.8, or an 11-fold increase. All nine vaccinees with a preexisting antibody titer of 20 or less seroconverted with a GMT of 316.8. One student with a prevaccine titer of 640 failed to seroconvert. Among the intactivated influenza vaccinees, there was no difference in antibody response correlated with sex or age.

None of the 28 placebo recipients experienced significant seroconversion. The pre-placebo GMT was 38.1 and the post-placebo GMT was 42.0.

There were no serious reactions to either the vaccines or the placebo (Table 2). Signs and symptoms usually were present for 1 to 2 days. The total score for the Alice strain vaccinees, including two 6-day periods after each of the vaccinations, was 315, or an average score of 2.1 per vaccinee. Thirty-six different vaccinees had at least one sign or symptom, or a rate of 48.6%. For the placebo group, the total score for the two 6-day rating periods was 118, or an average score of 2.1 per vaccinee. Seventeen of the 28 (60.7%) placebo recipients had some reaction score. The total for the inactivated influenza vaccine group was 79, or an average of 3.3. Nine of the 24 (37.5%) vaccinees developed some symptoms. However, none of the inactivated vaccine recipients had erythema or swelling or complained of pain at the site of injection.

TABLE 2. *Number of students with reactions*

| Sign or symptom | Alice vaccine[a] | | Inactivated vaccine[b] | | Placebo[c] | |
|---|---|---|---|---|---|---|
| | Mild | Moderate | Mild | Moderate | Mild | Moderate |
| Stuffy nose | 18 | 1 | 4 | 0 | 8 | 1 |
| Coryza | 23 | 3 | 6 | 0 | 10 | 2 |
| Cough | 6 | 0 | 3 | 0 | 1 | 0 |
| Sore throat | 11 | 0 | 4 | 0 | 10 | 0 |
| Headache | 9 | 2 | 4 | 1 | 4 | 1 |
| Gastrointestinal disturbance | 7 | 0 | 3 | 0 | 7 | 0 |
| Arthralgia | 6 | 0 | 2 | 0 | 1 | 0 |
| Myalgia | 7 | 0 | 7 | 1 | 2 | 0 |
| Fever | 8 | 0 | 3 | 0 | 6 | 0 |
| Avg score/subject | 2.1 | | 3.2 | | 2.1 | |

[a] 74 subjects graded for two 6-day periods.
[b] 24 subjects graded for one 6-day period.
[c] 28 subjects graded for two 6-day periods.

Temperature elevations were mild. Eight (5.4%) of the Alice vaccinees, six (10.7%) of the placebo recipients, and three (12.5%) of the inactivated vaccinees developed some fever during the rating period.

Almost all of the Alice strain vaccinees and the placebo recipients complained of a disagreeable taste several seconds after administration of the medicaments. This taste persisted for several minutes.

During the long-term follow-up, no influenza A occurred in the high school as determined by virus isolation and serological survey done on students with influenza-like illnesses.

## DISCUSSION

Immunoprophylaxis with currently available vaccines is the best method to counteract the disasterous effects of widespread influenza epidemics. However, protection afforded by these vaccines has been variable and short-lived (3). For this reason, selected annual immunization of high-risk groups rather than widespread immunization of the general population has been the modus operandi in this country (9). The problems stem from the constant antigenic shifting of the influenza viruses and relatively short period of protection afforded by the killed influenza vaccines.

Antigenic shifts of influenza virus can result in the circulation of a variant virus to which the population has no or little previous experience and lacks sufficient specific antibody to provide adequate protection. Production of new vaccines that incorporate the new variant is time consuming and can result in a lag period of an entire influenza season. To minimize delay in the manufacture of a new vaccine strain, recombinant strains have been developed. These strains recombine the newer antigenic characteristics, the hemagglutinin and neuraminidase, of the variant with an older strain that has been adopted to rapid growth in the laboratory. A recombinant can thus be made rapidly and can contain the contemporary antigenic determinants of the newly circulating influenza

strain. Such recombinants (some inactivated) have been made and are actively being evaluated for their protective efficacy (1, 5, 7, 14). Preliminary results indicate that they are indeed protective (7).

In the present study, a live, attenuated, recombinant influenza A vaccine administered intranasally was effective in stimulating significant seroconversion in students with undetectable or low preexisting serum antibodies. The vaccine was readily acceptable and produced no significant reactions. The reactions that did occur were minor and the same or lower in incidence than among the placebo and inactivated-vaccine recipients.

The overall antibody response in the two vaccine groups indicated that the inactivated vaccine produced a superior initial immunological response. Even greater seroconversion rates in students with high preexisting antibody titer could have been expected if an improved delivery system for the Alice vaccine had been used. Some students were unable to avoid swallowing the intranasally instilled drops. Spraying of the vaccine would probably be an improved method of vaccine delivery. Of importance would be the duration of the antibody response, which remains to be determined. With other viral vaccines, i.e., measles, the live, attenuated preparations have been associated with longer vaccine-induced antibody persistence than their inactivated counterparts (6). And finally, the intranasal route of administration of the Alice strain can be expected to produce a greater local immunoglobulin A secretory antibody response. Such a response is believed to be important in providing protection against respiratory tract viruses (12). Unfortunately, in this study collection of specimens to determine the immunoglobulin A antibody response was not permitted; however, in a small number of other subjects, Alice vaccination resulted in the development of local immunoglobulin A antibody (G. M. Schiff, unpublished data; A. Prinzi, personal communication).

The greater acceptability of a live, attenuated vaccine administered intranasally may have profound effect in achieving a satisfactory influenza immunization program. Part of the difficulty in present programs is the reluctance of patients and physicians to use preparations traditionally associated with a relatively high rate of reactions. Although the newer inactivated influenza vaccines are more purified and consequently have resulted in fewer reactions in children as well as adults, the onus on the inactivated influenza vaccines persists (10). Use of effective intranasal influenza vaccines should overcome this reluctance and even stimulate consideration for wider-scale immunization, including children, than is now recommended. Epidemiological studies have indicated that students play a significant role in the spread of influenza during community outbreaks (4). Some feel that immunization of children would be an efficient method to prevent influenza epidemics (2, 8).

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