JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1975, p. 403-409

Vol. 2, No. 5
Printed in U.S.A.

Attenuated Influenza A Vaccine (Alice) in an Adult Population: Vaccine-Related Illness, Serum and Nasal Antibody Production, and Intrafamily Transmission

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Received for publication 11 July 1975

Ninety-five healthy adults, ages 18 to 56 years, received two intranasal doses, 2 weeks apart, of a live, attenuated, influenza type A (H3N2) vaccine (an inhibitor-resistant recombinant strain of A/England/42/72 named "Alice"). Ninety-two persons were given placebos similarly. Ninety-three percent of 68 subjects with initial serum hemagglutination-inhibition (HI) titers of ≤1:40 to influenza A (H3N2) had a fourfold or greater antibody increase in postvaccination sera. Forty-four percent of 27 subjects with an initial HI titer of ≥1:80 had similar increases. Overall, 77% of vaccinees had fourfold or greater antibody titer increases. Vaccinees had geometric mean serum HI titers (GMT) of 1:26, 1:123, and 1:166 at 0, 14, and 30 days, respectively. The GMTs for placebos were 1:21, 1:22, and 1:21. Thirty-five vaccinees were examined for both serum and nasal antibody; 89% had significant increases in one or both. Nasal antibody response was directly related to the level of initial serum HI titer in that 83% of 12 persons with prevaccination HI titers of ≥1:80 showed significant nasal antibody rises, whereas only 61% of the remaining 23 subjects with prevaccination HI titers of ≤1:40 did so. The number and severity of clinical signs and symptoms reported by vaccinees and placebos did not differ significantly. The greatest differences noted between groups were for nasal congestion on days 0 to 6 (8.3%) and rhinitis on days 14 to 20 (5.9%). Four vaccinees shed Alice after primary vaccination, but viral titers were low (10 to 100 tissue culture-infective doses/ml). One member in each of 15 cohabiting male-female couples received Alice while the other received a placebo; one of the placebo members had significant increases in serum and nasal antibody, indicating a possible transmission.

Genetic recombinations of low growth-yielding strains of A/Hong Kong (A/HK) influenza with laboratory-adapted strains of A/PR8/34 have resulted in attenuated recombinants with the antigenicity of A/HK and the growth characteristics of A/PR8 (6). Strains of A/HK that are resistant to thermostable inhibitors present in normal serum, selected by passage of wild-type virus in embryonated eggs in the presence of heated horse or guinea pig serum, are attenuated for humans (6, 10). A live, attenuated, influenza type A (H3N2) vaccine known as "Alice" was developed by Recherche et Industrie Therapeutiques (R.I.T.), Rixensart, Belgium, a subsidiary of Smith Kline and French (SK&F) Laboratories (Philadelphia, Pa.), that is an inhibitor-resistant recombinant of A/England/42/72 and A/PR8/34. The purpose of the present study was to test this vaccine in humans to determine: (i) serum and nasal antibody response, (ii) vaccine-related illness, (iii) virus shedding, and (iv) capacity of Alice to spread between cohabiting male-female couples. A preliminary report has been given. (T. E. Minor, E. C. Dick, C. R. Dick, and S. L. Inhorn, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 315, 1975.)

MATERIALS AND METHODS

Experimental design. Coded (double-blind) preparations containing either Alice or a placebo were randomly administered intranasally to 187 volunteers beginning 27 November 1973 (day 0). A second dose was given on day 14. Blood samples for serologic analysis were collected on days 0, 14, and 30 to 41. Nasal washings for virus isolation and nasal antibody determination were obtained on days 2, 6, 16, 20, and 30 to 41.

Study group. Volunteers were solicited from: (i) personnel of the Wisconsin State Laboratory of Hygiene (Madison) and their spouses, and (ii) students from the Madison campus of the University of Wis-

consin and their spouses. Individuals with chronic bronchopulmonary, cardiovascular, or metabolic diseases were excluded. Seventy-two men and 115 women, ages 18 to 56 years, participated.

Administration of vaccine. Lyophilized material, consisting of either one dose of Alice ($10^{7.4}$ mean egg infective doses) or placebo (embryonated egg allantoic fluid), was reconstituted just before use in 0.5 ml of Gripovax (SK&F) (5% sucrose). The final preparation was administered intranasally, 0.25 ml in each nostril, with the subject in a supine position, head extended, during and for 1 min after vaccination. Subjects were instructed not to blow their noses for at least 15 min after inoculation.

Clinical surveillance. Volunteers were supplied with forms for daily recording of the following signs and symptoms: stuffy or runny nose, cough, sore throat, headache, general discomfort, pain in joints or muscles, fever, and chills. Severity was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). Percentage of volunteers reporting any given symptom and average severity of that symptom on days 0 to 6 and 14 to 20 were averaged for vaccinees and placebos, and differences were analyzed by Student's *t* test.

Nasal washings. Five milliliters of Hanks balanced salt solution with 0.25% gelatin, buffered to pH 7 with McIlvaine buffer (2), was instilled in each nostril while the subject was in a sitting position with the head in hyperextension. The fluid then was expelled into a sterile petri dish.

Isolation and identification of influenza virus. Primary rhesus monkey kidney cell cultures (RMK) (Grand Island Biological Co., Grand Island, N.Y.) were inoculated with 0.2 ml of nasal washing fluid. Cultures were tested for hemadsorption of guinea pig erythrocytes after 1, 2, and 4 weeks of incubation at 33 C. Isolates that were hemadsorption positive were tested for hemagglutination inhibition (HI) (1, 11) by A/England/42/72 antiserum (Center for Disease Control, Atlanta, Ga.). Influenza type A isolates were confirmed and tested for serum inhibitor resistance (C. Huygelen, R.I.T.).

Serum antibody assay. Sera were treated with receptor-destroying enzyme (Microbiological Associates, Bethesda, Md.) and then inactivated by heat (56 C, 30 min). Treated sera were tested by HI, using 4 hemagglutinating units of beta-propiolactone-inactivated influenza A strain MRC-7 (an earlier passage of the Alice strain) (SK&F) and 0.5% human type O erythrocytes.

Nasal antibody assay. Nasal washing fluids were homogenized in a Potter-Elvehjem tissue grinder and tested for presence of blood, using the Hemastix method (Hemastix, Ames, Iowa). Homogenates were prepared for nasal antibody assay (15) by centrifugation at 2,000 rpm for 20 min, dialysis against distilled water for 24 to 45 h, and approximately fivefold concentration by dialysis against 40 to 50% polyethylene glycol (Rugel Chemical, Irvington, N.J.). Concentrates were inactivated at 56 C (30 min) and then tested for neutralizing antibody to an Alice isolate from a vaccinee. Neutralization tests were performed in RMK tissue cultures and all serial specimens from a single individual were tested simultaneously. A rise in nasal antibody was considered significant if the titer increased from <1:2 to 1:2 or greater, or when any positive titer in the initial day 2 nasal washing increased by fourfold or more in a later specimen (5).

Nasal IgA assay. Unheated nasal washing concentrates were quantitatively analyzed for immunoglobulin A (IgA) by radial immunodiffusion (Hyland Laboratories, Costa Mesa, Calif., immunoplates with sensitivity range from 4 to 40 mg/100 ml). With this population it was not feasible to use either large nasal washing volumes (15) or nasal refluxing (10), and IgA concentrations were resultingly low, often below the level for accurate quantitation (4 mg/100 ml). Therefore, the nasal antibody neutralizing titers could not be adjusted to a standard IgA value and thus are reported in the tables without adjustment. However, the difference between the IgA levels in the 2-day and later convalescent nasal washings was usually small, and the IgA quantity was almost always higher in the day 2 specimens than in those taken subsequently (see Table 3 for IgA levels on sequential nasal washings).

RESULTS

Serum antibody response. Geometric mean serum antibody titers in 95 vaccinees increased 6.4-fold. These titers were 1:26, 1:123, and 1:166 after 0, 14, and 30 to 41 days, respectively. Corresponding mean titers for the 92 persons in the placebo group were 1:21, 1:22, and 1:21.

Serum antibody responses were greatest in persons with initial HI titers of ≤1:40 (Table 1). All 18 subjects with prevaccination titers of <1:10 had at least fourfold rises. Ninety-three percent of 68 vaccinees with ≤1:40 initial titers had ≥fourfold rises. In contrast, only 44% of 27 persons with >1:40 initial titers had significant antibody increases. Overall, 73 of the 95 vaccinees (77%) had ≥fourfold rises and 48 of these increments were eightfold or more. All but 11 of the 73 seroconversions occurred within the first 14 days of the study (prior to the second dose of Alice).

Nasal antibody response. Concentrated Hemastix-negative nasal washings from 35 vaccinees were tested for the presence of neutralizing antibody in day 2 and day 16 to 41 specimens (Table 2). The proportion of individuals with low and high initial serum antibody titers in this group of 35 is similar to that of the original 95 vaccinees. Twenty-four vaccinees (68%) demonstrated a significant increase in nasal antibody. The highest percentage of persons with significant increases, 83%, was in those with initial serum antibody titers of 1:80 and above.

Some vaccinees developed nasal antibody without concomitant significant increases in serum antibody, and the reverse was also seen. (As examples of both circumstances, see subjects SM, JW, ER, BR, MC, and LP in Table 3.)

TABLE 1. *Relationship between initial serum HI antibody titers and antibody rises observed in 95 healthy adult volunteers 30 to 41 days after intranasal administration of live, attenuated, influenza type A (H3N2) vaccine (Alice strain)*[a]

Initial titer	No. with titer	No. of subjects with rise in serum antibody						Subjects with significant (≥fourfold) rises	
		No change	Two-fold rise	Four-fold rise	Eight-fold rise	16-fold rise	>16-fold rise		
<1:10	18	0	0	5	4	6	3	18	(100%)
1:10	18	0	2	3	3	9	1	16	(89%)
1:20	21	0	2	6	8	2	3	19	(91%)
1:40	11	0	1	3	4	2	1	10	(91%)
Subtotal	68	0	5	17	19	19	8	63	(93%)
1:80	12	1	4	5	1	1	0	7	(58%)
1:160	6	1	3	2	0	0	0	2	(33%)
1:320	7	3	3	1	0	0	0	1	(14%)
1:640	1	1	0	0	0	0	0	0	(0%)
≥1:1,280	1	1	0	0	0	0	0	0	(0%)
Subtotal	27	7	10	8	1	1	0	10	(44%)
Total	95	7	15	25	20	20	8	73	(77%)

[a] A second dose of vaccine was administered 14 days after the first.

TABLE 2. *Relationship between prevaccination serum HI titer and nasal antibody rises in 35 volunteers 16 to 41 days after intranasal administration of Alice vaccine*[a]

Serum antibody		No. of subjects with nasal antibody on day 2[b]	No. of subjects with a rise in nasal antibody titer				Total subjects with a rise in nasal antibody titer	
Prevaccination titer	No. with titer		No change	Two- to threefold	Four- to sixfold	≥Eightfold		
<1:10	11	1	5	4	1	1	6	(55%)
1:10	3	1	1	2	0	0	2	(66%)
1:20	5	0	2	1	1	1	3	(60%)
1:40	4	1	1	1	2	0	3	(75%)
Subtotal	23	3	9	8	4	2	14	(61%)
1:80	5	1	0	2	1	2	5	(100%)
1:160	5	3	2	0	2	1	3	(60%)
1:320	1	0	0	0	0	1	1	(100%)
1:640	1	0	0	0	0	1	1	(100%)
Subtotal	12	4	2	2	3	5	10	(83%)
Total	35	7	11	10	7	7	24	(68%)

[a] A second dose of vaccine was administered 14 days after the first.

[b] Prevaccination nasal washes were not taken. No day 2 specimen nasal wash titers were greater than 1:3. Where an antibody increase occurred (five of seven cases), it ranged from four- to 12-fold.

Only 24 (71%) showed significant serum antibody rises, but an additional six vaccinees developed nasal antibody only. Therefore, the vaccine stimulated a significant immunologic response in 31 (89%) of this 35 member group. Most of the vaccinees (four of the six) who developed only nasal antibody had initial serum HI titers of 1:80 or greater; this group had a very low serologic conversion rate (see Table 1).

Serial specimens from 19 vaccinees were tested for neutralizing antibody (Table 3). There was no evidence for any nasal antibody increase before the second dose was administered at day 14. Also, no vaccinee with initial serum antibody levels of 1:10 or less had increased nasal antibody before 30 days. However, five persons with higher initial serum antibody levels had increased nasal antibody on day 16, either as a result of the initial vaccination or of the booster. There was no evidence of a gradual nasal antibody increase over the period of observation.

Clinical reactions. The average number of vaccinees who experienced a particular clinical sign or symptom on days 0 to 6 and 14 to 20 was not significantly ($P > 0.05$) different from responses in the placebo group (Table 4). The greatest differences noted between groups were for nasal congestion on days 0 to 6 (8.3%) and rhinitis on days 14 to 20 (5.9%). Vaccine and

TABLE 3. *Relationship between the development of serum HI antibody and of nasal washing neutralizing antibody in volunteers given Alice vaccine intranasally*[a]

Subjects	Serum HI antibody titers			≥Four-fold serum antibody rises	Days after primary vaccination					Nasal antibody increases
	Pre-vaccination	14 days	30 to 41 days		2	6	16	20	30–41	
DB	<10	160	640	+	<2 (4)[b]	<2	<2	<2	6 (5)	+
JL	<10	20	20	+	<2 (4)	<2	<2	<2	3 (4)	+
RK	<10	40	160	+	<2 (6)	<2(<4)	<2(<4)	<2 (4)	4(<4)	+
SM	<10	20	20	+	<2	<2	<2	<2	ND[c]	−
JW	<10	20	40	+	2	<2	<2	<2	<2	−
ER	10	10	20	−	<2 (9)	<2 (5)	<2	<2 (9)	3	+
RS	10	80	160	+	<2 (4)	<2	<2	<2	3(<4)	+
NA	20	160	320	+	<2 (5)	<2 (5)	6 (8)	16 (4)	3 (4)	+
KR	20	160	160	+	<2(<4)	<2 (4)	<2(<4)	<2(<4)	6(<4)	+
BR	20	160	80	+	<2	<2	<2	<2	<2	−
MC	40	80	80	−	<2(<4)	<2	<2	<2	3(<4)	+
KP	40	≥1280	≥1280	+	<2 (4)	<2(<4)	6(<4)	4(<4)	3(<4)	+
ME	80	80	≥1280	+	<2 (4)	<2	<2	<2 (4)	11 (4)	+
WF	80	160	320	+	<2(<4)	<2	<2	<2	6(<4)	+
TW	80	320	320	+	<2(<4)	<2	<2	<2	3(<4)	+
GG	80	640	640	+	2 (8)	<2 (13)	23 (4)	ND	11(<4)	+
MK	160	320	640	+	3 (9)	ND	4(<4)	16 (10)	16 (4)	+
LP	160	320	640	+	<2	<2	<2	<2	<2	−
JC	320	≥1280	≥1280	+	<2(<4)	<2(<4)	11(<4)	6(<4)	8(<4)	+

[a] A second intranasal dose of vaccine was given at 14 days.
[b] Nasal antibody titers. Parentheses show IgA in mg/100 ml.
[c] ND, Not done.

TABLE 4. *Clinical signs/symptoms reported by 187 adult volunteers during a 6-day period after primary (day 0) and secondary (day 14) intranasal administration of live, attenuated, influenza type A (H3N2) vaccine (Alice strain) or placebo*[a]

Clinical signs/symptoms	Average of subjects with clinical signs/symptoms (%)					
	Days 0 to 6			Days 14 to 20		
	Vaccine group	Control group	Difference[b]	Vaccine group	Control group	Difference[b]
Nasal congestion	22.9	14.6	8.3	17.7	14.6	3.1
Rhinitis	19.4	19.9	−0.5	17.8	11.9	5.9
Cough	13.2	12.7	0.5	11.2	9.2	2.0
Sore throat	6.5	6.6	−0.1	7.8	6.6	1.2
Headache	11.5	12.4	−0.9	5.1	7.9	−2.8
Malaise	5.9	6.4	−0.5	4.3	5.6	−1.3
Arthralgia	0.8	3.1	−2.3	1.3	3.0	−1.7
Myalgia	2.7	5.4	−2.7	3.5	5.3	−1.8
Chills	2.6	3.7	−1.1	0.3	3.8	−3.6
Temperature (>37.8 C)	0.3	0	0.3	1.1	0	1.1

[a] Ninety-five vaccinees and 92 placebos.
[b] Percentage in vaccine group minus percentage in placebo group. None of the differences were statistically significant at the $P = 0.05$ level.

placebo subjects had identical overall mean symptom scores of 1.4 (mild to moderate) (data not shown).

Shedding of Alice. Viruses indistinguishable from Alice (C. Huygelen, personal communication) were isolated from four of the 95 vaccinees (Table 5). All four shed Alice on day 2 and one of the four also shed the virus on day 6. The titers in RMK of day 2 nasal washings ranged from 10 to 100 mean tissue culture infective doses ($TCID_{50}$)/ml. Shedding was accompanied by mild clinical signs and symptoms and by significant serum antibody rises.

Transmissibility of Alice. One partner in

TABLE 5. *Shedding of vaccine virus in adult volunteers after primary (day 0) and secondary (day 14) intranasal administration of live, attenuated, influenza type A (H3N2) vaccine (Alice strain)*[a]

Subject	Serum HI antibody		Day of shedding	Titer[b] of isolate	Clinical signs/symptoms
	Day 0	Day 30			
2,034	1:10	1:160	2	100	Rhinitis
2,050	1:20	1:160	2, 6	18	Rhinitis, headache
2,059	<1:10	1:160	2	32	Nasal congestion
2,141	<1:10	1:320	2	10	Rhinitis

[a] Nasal washing specimens obtained on days 0, 6, 14, and 20.
[b] Number of $TCID_{50}$ (primary RMK)/milliliter, day 2.

each of 15 married couples received Alice while the other received a placebo. One of the 15 placebo members had an initial HI titer of 1:160, whereas five had 1:20 to 1:40 and nine had <1:10. Alice was not isolated from any of the vaccinated partners.

One placebo partner had a fourfold serum antibody titer rise to Alice (from 1:40 to 1:160). Similar values for these serum specimens were obtained in duplicate coded samples sent to R.I.T. (C. Huygelen, personal communication). Nasal antibody titers of <1:2, 1:4, 1:2, and <1:2 were present after 0, 6, 16, and 20 days, respectively. A myxovirus was recovered from this subject on day 2, but the agent was identified as a parainfluenza type 4. Virus shedding was accompanied by rhinitis, cough, sore throat, headache, malaise, and myalgia. Serum neutralization antibody to the parainfluenza 4 isolate rose from <1:14 to 1:113.

DISCUSSION

Serum antibody responses observed in volunteers vaccinated with live, attenuated, Alice influenza virus were comparable to those shown by others to be produced by highly potent inactivated, parenterally administered vaccines. Wenzel et al. (15) tested such a vaccine and observed at least fourfold serum antibody rises in all volunteers with <1:10 initial titers, in 62% of those with <1:160 initial titers, and a geometric mean serum antibody increase for all subjects from 1:65 to >1:212. Vaccination with Alice also induced ≥fourfold titer rises in all subjects with <1:10 initial titers and in 77% of all subjects and resulted in a geometric mean titer rise of 1:26 to 1:166 for all vaccinees.

Alice vaccine had remarkably low virulence for human subjects in that clinical reactions in vaccinees were statistically indistinguishable from symptoms reported by placebo subjects. Current inactivated vaccines also have low toxicity for humans (4, 6, 15).

A prominent feature of the Alice vaccine was its ability to induce good nasal antibody responses. Significant rises in neutralizing antibody were found in 68% of 35 vaccinees tested. Alice was especially effective in stimulating nasal antibody production in those persons with high (>1:80) prevaccination serum HI titers. In contrast, parenterally administered inactivated influenza vaccines often are poor inducers of nasal antibody. Fulk et al. (3) found subcutaneous vaccination completely ineffective in stimulating nasal antibody in 64 adults, and Kasel et al. (5) never found nasal antibody titers above 1:2 in 27 men similarly vaccinated. Ruben and Jackson (12) found that only one of nine persons given a subunit vaccine parenterally produced significant serum antibody titer rises. On the other hand, Wenzel and his associates (15) found that a potent parenterally administered vaccine produced nasal antibody responses in nine of 18 men. Nasal instillation of inactivated vaccines has met with mixed results (3, 4, 14). Some have reported it ineffective in mobilization of serum (3, 4) and nasal (3) antibody, whereas others (14) have found it to be relatively effective in both these areas.

Alice virus was recovered from four of 95 subjects on the second day after vaccination and from one of these four on day 6 as well. The quantity of virus in the day 2 nasal washings was from 10 to 100 $TCID_{50}$. This is a considerably lower rate and level of virus shedding than was reported by Murphy et al. (9) for the temperature-sensitive attenuated vaccine candidate strain, influenza A (H3N2)-ts-1[E]. On the second day after infection, they isolated virus from eight of 17 vaccinees, and four of the eight positive nasal specimens had virus in concentrations from approximately 10^3 to 10^6 $TCID_{50}$/ml. Theoretically, some of these persons could have been infectious for others on day 2 as the mean human infectious dose for the ts-1[E] strain is 10^5 $TCID_{50}$ (9). The amount of virus in the nasal washes from the Alice vaccinees was much lower than the mean human infectious doses for Alice, which is from 10^6 to 10^7 mean egg infective doses (R. G. Douglas, personal communication). Although these levels would not be expected to produce cross infections, it is possible that our method of virus detection was not

optimal. Douglas (personal communication) has suggested that embryonated eggs may be more sensitive than RMK for isolation of Alice from clinical specimens. On the other hand, eggs do not appear to be more sensitive than RMK in detecting vaccine virus. We titrated in triplicate the Alice vaccine stated by SK&F to contain $10^{7.4}$ mean egg infective doses and demonstrated RMK titers ranging from $10^{7.0}$ to $10^{7.5}$ $TCID_{50}$ (authors' unpublished data).

Despite the apparently low rate and level of Alice virus shedding, there was serologic evidence (a significant increase in serum and nasal antibody) that transmission may have occurred in one of 15 cohabiting couples. It is our view, however, that the evidence for transmission is not conclusive. A parainfluenza 4 virus was recovered from this person during the period of acute illness, coincident with the antibody rise to Alice. Although we are not aware of any supporting evidence for such a concept, it seems possible that some nonspecific stimulation of influenza antibody could have occurred coincidentally with the parainfluenza 4 infection. Lack of communicability of live influenza vaccines is a very important attribute (6), and it is hoped that others will attempt confirmatory transmission studies under similar conditions of intimacy.

Two evaluations of Alice vaccine have just been published. Schiff et al. (13), using high school children, compared Alice vaccine with parenterally given inactivated influenza vaccine, and with a placebo for vaccine reaction and ability to stimulate serum antibody. They noted minimal clinical reactions to both vaccines, and their serologic conversion rates and titers for Alice vaccination were similar to those reported for our own population. Miller et al. (7) studied Alice vaccine in an elderly population, comparing its effectiveness to parenterally administered inactivated vaccine. For ethical reasons, no placebo was included. The Alice vaccine reactions, although not severe, were considerably greater than noted in our population or in the high school students studied by Schiff and his associates (13). Both serum and nasal antibody production were found to be less efficiently elicited by Alice vaccine than by the killed virus vaccine.

Viral respiratory infections, including influenza, can provoke attacks of asthma in susceptible persons (8). We have found that Alice does not provoke airway obstruction nor other serious respiratory symptoms in asthmatic patients, but it does evoke satisfactory rises in serum antibody titers (W. W. Storms, E. C. Dick, and W. W. Busse, J. Allergy Clin. Immunol.).

The present study showed that the Alice vaccine produced very few clinical reactions in its recipients and was highly immunogenic both with respect to production of serum and nasal antibody. Whether this apparently very promising attenuated vaccine (promising at least for high school and young to middle age adult populations) will effectively protect against natural illness must of course await the results of controlled field trials.

ACKNOWLEDGMENTS

The technical assistance of C. I. Allen, D. J. D'Alessio, D. E. Docherty, S. A. Gould, J. A. Peterson, and M. M. Schultz is gratefully acknowledged.

This study was supported by Smith Kline and French Laboratories, Philadelphia, Pa., and by Public Health Service grant no. AI-10, 404-01 from the National Institute of Allergy and Infectious Diseases.

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