

## THE EFFICACY OF LIVE ATTENUATED, COLD-ADAPTED, TRIVALENT, INTRANASAL INFLUENZAVIRUS VACCINE IN CHILDREN

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### ABSTRACT

**Background** Influenzavirus vaccine is used infrequently in healthy children, even though the rates of influenza in this group are high. We conducted a multicenter, double-blind, placebo-controlled trial of a live attenuated, cold-adapted, trivalent influenzavirus vaccine in children 15 to 71 months old.

**Methods** Two hundred eighty-eight children were assigned to receive one dose of vaccine or placebo given by intranasal spray, and 1314 were assigned to receive two doses approximately 60 days apart. The strains included in the vaccine were antigenically equivalent to those in the inactivated influenzavirus vaccine in use at the time. The subjects were monitored with viral cultures for influenza during the subsequent influenza season. A case of influenza was defined as an illness associated with the isolation of wild-type influenzavirus from respiratory secretions.

**Results** The intranasal vaccine was accepted and well tolerated. Among children who were initially seronegative, antibody titers increased by a factor of four in 61 to 96 percent, depending on the influenza strain. Culture-positive influenza was significantly less common in the vaccine group (14 cases among 1070 subjects) than the placebo group (95 cases among 532 subjects). The vaccine efficacy was 93 percent (95 percent confidence interval, 88 to 96 percent) against culture-confirmed influenza. Both the one-dose regimen (89 percent efficacy) and the two-dose regimen (94 percent efficacy) were efficacious, and the vaccine was efficacious against both strains of influenza circulating in 1996–1997, A(H3N2) and B. The vaccinated children had significantly fewer febrile illnesses, including 30 percent fewer episodes of febrile otitis media (95 percent confidence interval, 18 to 45 percent;  $P < 0.001$ ).

**Conclusions** A live attenuated, cold-adapted influenzavirus vaccine was safe, immunogenic, and effective against influenza A(H3N2) and B in healthy children. (N Engl J Med 1998;338:1405-12.)

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**I**NFLUENZA A and B viruses are among the most common causes of respiratory tract illnesses that bring children to medical care, and influenza is a major cause of lower respiratory tract illness in young children.<sup>1</sup> The annual incidence of influenza infection in children may exceed 30 percent,<sup>2</sup> and children are believed to be important in the spread of influenza in the community.<sup>3</sup>

Despite the availability of inactivated influenzavirus vaccine for children, the vaccine is used infrequently in this age group.<sup>4</sup>

Live attenuated, cold-adapted, trivalent influenza virus vaccine that is given intranasally may represent a convenient and effective approach to the prevention of influenza in children. The vaccine antigens are updated annually by genetic reassortment techniques that substitute genes encoding the hemagglutinin and neuraminidase antigens from contemporary influenza A and B viruses for those in the master attenuated strains. The derivation of the vaccine master strains, the reassortment process, and previous clinical evaluation have been the subject of several reviews.<sup>5-8</sup> The purpose of the present study was to determine the efficacy in children of the live attenuated, cold-adapted, trivalent influenzavirus vaccine administered by nasal spray.

### METHODS

#### Vaccine and Placebo

Cold-adapted, trivalent influenzavirus vaccine was supplied by Aviron (Mountain View, Calif.), frozen in single-dose intranasal applicators as described below. The mean tissue-culture infective dose of each of the three attenuated strains included in the vaccine was  $10^{6.7}$ . The strains chosen matched the antigens recommended for the inactivated influenzavirus vaccine by the Food and Drug Administration for the 1996–1997 influenza season. Vaccine reassortants were produced as previously described and included influenza A/Texas/36/91-like (H1N1), A/Wuhan/359/95-like (H3N2), and B/Harbin/7/94-like viruses in egg al-

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allantoic fluid with sucrose, phosphate, and glutamate.<sup>9-13</sup> The vaccine was stored frozen at  $-20^{\circ}\text{C}$ ; thawed vaccine could be stored for up to eight hours in the refrigerator (temperature,  $2$  to  $8^{\circ}\text{C}$ ) before use. The placebo consisted of egg allantoic fluid containing sucrose, phosphate, and glutamate and was indistinguishable in appearance and smell from the vaccine. The spray applicator consisted of a syringe-like device that was calibrated and divided for the delivery of two 0.25-ml aliquots (one per nostril) as a large-particle aerosol, for a total delivered volume of 0.5 ml of study vaccine or placebo.

Vaccine and placebo were randomly assigned sequential vaccination numbers with a block size of six. The randomization sequence was incorporated into the preparation and labeling of materials, and each eligible child received the next available study number at a site, ensuring proper randomization. Each child underwent randomization individually.

### Subjects

Healthy children who were 15 to 71 months of age at the time of recruitment were enrolled in the study. Informed consent was obtained from a parent or guardian. Children with a history of clinically significant hypersensitivity to eggs were excluded from the study, as were those with underlying chronic illnesses, for whom the inactivated vaccine would be recommended. Subjects scheduled to receive two doses of vaccine received the first dose between August 21, 1996, and October 23, 1996, and the second dose between October 15, 1996, and January 11, 1997. Subjects in the one-dose cohort were enrolled and vaccinated from September 30, 1996, through December 5, 1996.

### Study Design

The study was prospective, randomized, double blind, placebo controlled, and multicenter in design. The primary efficacy end point was the first episode of culture-confirmed influenza for subjects who became ill 28 days or more after the receipt of the first dose of vaccine or placebo or at any time after the receipt of the second dose during the influenza season. The subtype-specific efficacy of the vaccine was evaluated, and the analyses included all first cases of influenza A or B. The subjects were randomly assigned in a 2:1 ratio to receive vaccine or placebo and were then monitored throughout the subsequent influenza season. The vaccine or placebo was given as either a one-dose or two-dose regimen. Six centers used the two-dose regimen alone. Two of the sites used primarily the one-dose regimen, and two other sites used primarily the two-dose regimen, but late in the vaccination season both switched to the one-dose regimen.

Two hundred three subjects participated in a substudy of immunogenicity to characterize strain-specific antibody responses to the vaccine. This cohort consisted of approximately the first 21 children recruited at each site for the efficacy study. The subjects had blood drawn before receiving each dose and again four weeks after the second dose.

The second dose of vaccine was given approximately 60 days after the first dose, with a window period of  $\pm 14$  days. However, if subjects had an intercurrent illness or for some reason could not receive the second dose within the target window, they were given the second dose as soon as possible thereafter.

### Postvaccination Reactions

To evaluate whether there were any side effects of vaccination, the parent or guardian of each subject was given a digital thermometer and asked to record on a diary card the subject's temperature and the occurrence of specific symptoms, including decreased activity, irritability, runny nose or nasal congestion, sore throat, cough, headache, muscle aches, chills, and vomiting, daily for 10 days after each vaccination. Serious adverse events occurring within 42 days of vaccination and vaccine-related serious adverse events occurring at any time during the study were recorded by study personnel.

### Surveillance for Influenza and Case Definitions

Parents were contacted by telephone every two to three weeks until the beginning of an influenza outbreak in their community. Thereafter, weekly contact was made with the families to remind parents to notify study personnel if the subjects had symptoms suspected to be caused by influenza; these included fever, runny nose or nasal congestion, sore throat, cough, headache, muscle aches, chills, vomiting, suspected or confirmed otitis media, decreased activity, irritability, wheezing, shortness of breath, and pulmonary congestion. A report of any of these symptoms or signs was to result in a culture for viruses. The staff at the study sites attempted to collect viral-culture specimens from symptomatic subjects within four days after the onset of any illness. Tissue cultures of rhesus-monkey-kidney cells were inoculated with fresh respiratory secretions within four hours after collection or as soon as possible thereafter in order to cultivate influenzaviruses.

A case of influenza was defined as any illness detected by active surveillance (as described above) that was associated with a positive culture for wild-type influenzavirus. Positive viral cultures obtained within 28 days after the first or second dose of vaccine were phenotyped to determine whether the isolated viruses were wild-type influenza or one of the strains in the vaccine, indicating shedding of vaccine virus.

As part of active surveillance for symptoms and signs of influenza, reports of illness included whether or not the child was seen by the primary care provider; the provider's diagnosis and treatment were also recorded. The diagnoses included otitis media with or without concomitant fever and antibiotic treatment. A case of febrile otitis media was defined as any diagnosis of otitis media made by a health care provider that was associated with fever (whether or not the temperature was documented with a thermometer).

### Serologic Studies

Serum samples were obtained from the cohort in the immunogenicity substudy, stored at  $-20^{\circ}\text{C}$ , and assayed for the presence of hemagglutination-inhibiting antibodies to the three viral strains contained in the vaccine.<sup>14</sup> Antibody titers of  $\leq 1:4$  were considered to represent seronegativity.

### Statistical Analysis

Data were monitored on site and were entered both on site and at a central facility. Reports of adverse events were coded with COSTART (Coding Symbols for Thesaurus of Adverse Reaction Terms)<sup>15</sup> by Phoenix International (Irvine, Calif.). Statistical analyses were performed with SAS version 6.12<sup>16</sup> and StatXact 3<sup>17</sup> software. Point estimates of efficacy were calculated with the following equation:  $100 \times (1 - \text{relative risk}) = 100 \times (1 - P_v/P_p)$ , where  $P_v$  and  $P_p$  are the observed proportions of cases in vaccine recipients and placebo recipients, respectively. Koopman's method for the ratio of binomials<sup>17</sup> was used to estimate 95 percent confidence intervals. We used a logistic generalized estimation equation with an exchangeable covariance matrix to rule out the possibility of an effect within families on the results, since in many cases more than one family member was included in the study. Two-sided P values are reported. For the analysis of vaccination reactions, P values were adjusted separately for each vaccination and symptom with Bonferroni's method.<sup>17</sup> Confidence intervals for the ratio of mean episodes were computed with Poisson regression, with an offset reflecting the length of time available for observation. The percent reduction in the mean number of episodes was calculated with the following equation:  $100 \times (1 - \text{ratio of mean episodes})$ .

## RESULTS

Enrollment began in August 1996, and a total of 1314 children were enrolled in the two-dose cohort and 288 in the one-dose cohort. Demographic data on the participants are summarized in Table 1. There

**TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF THE CHILDREN IN THE STUDY.\***

CHARACTERISTIC	VACCINE GROUP (N=1070)	PLACEBO GROUP (N=532)
Sex — no. (%)		
Female	571 (53)	272 (51)
Male	499 (47)	260 (49)
Race or ethnic group — no. (%)		
White	906 (85)	449 (84)
Black	93 (9)	52 (10)
Hispanic	38 (4)	17 (3)
Asian	10 (1)	4 (1)
Other	23 (2)	10 (2)
Age — mo	43.0±16.6	41.5±16.5
Primary caretaker works outside home — no. (%)	524 (49)	265 (50)
Enrollment in day care or preschool — no. (%)		
0 days/wk	378 (35)	193 (36)
<1 day/wk	6 (<1)	4 (1)
1 day/wk	32 (3)	25 (5)
2 days/wk	113 (11)	59 (11)
3 days/wk	146 (14)	59 (11)
4 days/wk	46 (4)	25 (5)
5 days/wk	345 (32)	166 (31)
>5 days/wk	4 (<1)	1 (<1)
Mean days/wk	2.4±2.1	2.3±2.1
Household makeup		
No. of adults	2.1±0.6	2.1±0.6
No. of children	2.6±1.2	2.6±1.1
No. of household members in study — no. (%)		
1	513 (48)	258 (48)
2	463 (43)	221 (42)
3	86 (8)	49 (9)
4	8 (1)	4 (1)

\*Plus-minus values are means ±SD. Because of rounding, not all percentages total 100.

were no statistically significant differences in age, sex, race, day-care use, or household makeup between the vaccine and placebo groups.

### Safety

Ninety-seven percent of the children enrolled in the two-dose cohort received both doses of vaccine or placebo. The second dose was withheld from two children who had adverse reactions to the first dose; both these children were placebo recipients. One of these children had hives beginning four days after receiving the first dose, and wheezing developed in the other after the first dose. Forty children did not receive the second dose for other reasons, including withdrawal of consent (18 children), intercurrent illness (7), protocol violation or withdrawal of the child by an investigator (12), and loss to follow-up or departure from the area (3).

Some vaccinated children had transient, minor symptoms of respiratory tract illness. The incidence of rhinorrhea or nasal congestion, fever, and decreased activity after the first dose of vaccine or placebo is summarized in Table 2. Rhinorrhea or nasal

congestion (on days 2, 3, 8, and 9), fever (on day 2), and decreased activity (on day 2) were significantly associated with the receipt of vaccine. The fever was short lived (mean duration, 1.4 days) and low grade (mean temperature, 38.2°C [100.7°F] among the children with fever). Relatively high fevers occurred infrequently in both groups: there were 20 children with temperatures of 38.3°C (101°F) or higher on day 2 in the vaccine group and 4 in the placebo group (P=0.08).

On any individual day from day 1 through day 10, there were no significant differences between groups in any other symptoms or signs that were evaluated, including cough, headache, sore throat, irritability, chills, vomiting, and muscle aches. However, vomiting was reported in more children in the vaccine group than in the placebo group at some time on days 1 through 10 after the first dose (unadjusted P=0.03). Evaluation of 59 COSTART codes identified abdominal pain as being significantly associated with the first dose of vaccine (occurring in 19 children), as compared with the first dose of placebo (1 child). The abdominal pain was brief (mean duration, 3.0 days) and in 16 cases was judged as mild in intensity.

After the second dose, there were no significant differences between the groups in the occurrence of any sign or symptom on any day. Four serious adverse events occurred in the vaccine group within 42 days of vaccination (*Staphylococcus aureus* foot infection, abdominal pain, motor vehicle accident, and dehydration), and one occurred in the placebo group (hospitalization for revision of ventriculoperitoneal shunt); in no instance did the investigators attribute the adverse event to the vaccination.

### Immunogenicity

Antibody responses to vaccine and placebo are summarized in Table 3. Among the 203 children in the immunogenicity substudy, 67 percent were seronegative for influenza A(H1N1) before vaccination, 47 percent were seronegative for influenza A(H3N2), and 67 percent were seronegative for influenza B. There was no significant difference in the distribution of seronegative children between the vaccine and placebo cohorts. Younger children were more likely to be seronegative than older children. Among one-year-olds and two-year-olds, for example, only 29 percent had antibodies to influenza A(H3N2) before vaccination, as compared with 70 percent of children three years of age or older.

The vaccine was highly immunogenic for the influenza A(H3N2) and B subtypes after the first dose. As in previous studies, more than one dose was required to induce serum antibodies to the influenza A(H1N1) component in the majority of children.<sup>18,19</sup> Overall, after two doses of vaccine, 61 percent of initially seronegative children had antibodies to influ-

**TABLE 2.** INCIDENCE OF RHINORRHEA OR NASAL CONGESTION, FEVER, AND DECREASED ACTIVITY AFTER THE FIRST DOSE OF LIVE ATTENUATED, COLD-ADAPTED INFLUENZAVIRUS VACCINE OR PLACEBO.\*

DAY AFTER DOSE	RHINORRHEA OR NASAL CONGESTION		RELATIVE RISK (95% CI)	ADJUSTED P VALUE†	FEVER‡		RELATIVE RISK (95% CI)	ADJUSTED P VALUE†	DECREASED ACTIVITY		RELATIVE RISK (95% CI)	ADJUSTED P VALUE†
	VACCINE GROUP	PLACEBO GROUP			VACCINE GROUP	PLACEBO GROUP			VACCINE GROUP	PLACEBO GROUP		
	%				%				%			
1	15	16	1.0 (0.7–1.2)	1.0	1.8	1.4	1.3 (0.5–2.8)	1.0	2.2	3.1	0.7 (0.4–1.3)	1.0
2	27	18	1.5 (1.2–1.8)	0.001	6.5	1.6	4.1 (2.0–8.3)	<0.001	6.0	2.1	2.8 (1.5–5.2)	0.008
3	30	20	1.5 (1.2–1.8)	<0.001	3.6	2.4	1.5 (0.8–2.9)	1.0	4.5	2.7	1.6 (0.9–2.9)	0.9
4	26	21	1.2 (1.0–1.5)	0.6	2.1	2.7	0.8 (0.4–1.5)	1.0	3.5	2.5	1.4 (0.7–2.6)	1.0
5	24	24	1.0 (0.8–1.2)	1.0	2.0	2.8	0.7 (0.4–1.4)	1.0	2.8	2.5	1.1 (0.6–2.1)	1.0
6	24	22	1.1 (0.9–1.3)	1.0	2.0	1.9	1.0 (0.5–2.1)	1.0	2.3	1.4	1.7 (0.8–3.9)	1.0
7	26	22	1.2 (1.0–1.5)	0.7	1.3	1.8	0.7 (0.3–1.6)	1.0	2.3	0.8	3.0 (1.1–8.2)	0.3
8	30	22	1.4 (1.1–1.6)	0.01	1.2	1.4	0.9 (0.3–2.1)	1.0	2.6	1.0	2.7 (1.1–6.7)	0.3
9	29	21	1.3 (1.1–1.6)	0.02	1.6	1.2	1.3 (0.6–3.9)	1.0	2.0	1.0	2.1 (0.8–5.3)	1.0
10	26	20	1.3 (1.1–1.6)	0.1	1.2	1.4	0.9 (0.4–2.6)	1.0	1.9	1.2	1.6 (0.7–3.8)	1.0
Any day (1–10)	58	47	1.2 (1.1–1.4)	<0.001	15	11	1.4 (1.0–1.8)	0.05	16	12	1.3 (1.0–1.7)	0.06

\*Values represent all reported results; more than 85 percent of the children had complete reporting of safety data. There were no significant differences in any of the variables, including rhinorrhea or nasal congestion, fever, and decreased activity on any day after the second dose. CI denotes confidence interval.

†P values were adjusted by Bonferroni's method to account for multiple comparisons for days 1 through 10. P values for any day (1–10) were not adjusted.

‡Fever was defined as an axillary temperature above 37.6°C (99.6°F), an oral temperature above 37.7°C (100.0°F), or a rectal temperature above 38.1°C (100.6°F).

**TABLE 3.** HEMAGGLUTINATION-INHIBITING ANTIBODY RESPONSES AFTER ONE OR TWO DOSES OF LIVE ATTENUATED, COLD-ADAPTED INFLUENZAVIRUS VACCINE OR PLACEBO.

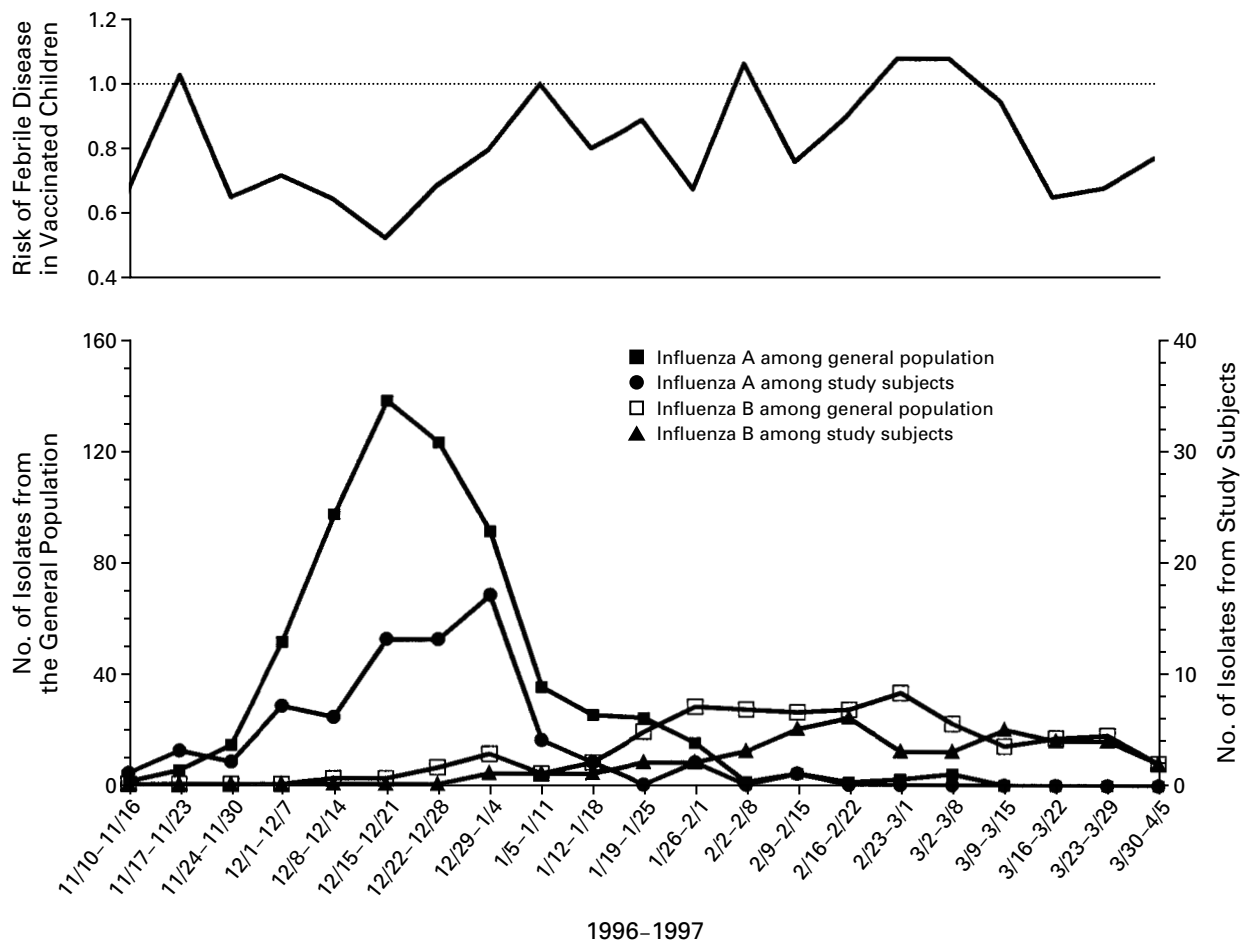
STUDY GROUP AND VIRUS TYPE	NO. TESTED	NO. SERONEGATIVE BEFORE VACCINATION*	NO. OF SERONEGATIVE CHILDREN WITH ANTIBODY RESPONSE†			GEOMETRIC MEAN ANTIBODY TITER AFTER DOSE 2	
			DAY OF DOSE 1 TO BEFORE DOSE 2‡	DAY OF DOSE 2 TO 28 DAYS AFTERWARD§	DAY OF DOSE 1 TO 28 DAYS AFTER DOSE 2	AMONG INITIALLY SERONEGATIVE CHILDREN	AMONG ALL CHILDREN TESTED
			no./total no. tested (%)				
Vaccine							
A(H1N1)	136	89	14/86 (16)	33/60 (55)	45/74 (61)	9	19
A(H3N2)	136	66	59/64 (92)	3/4 (75)	54/56 (96)	34	44
B	136	93	80/91 (88)	6/8 (75)	75/78 (96)	25	26
Placebo							
A(H1N1)	67	47	0/45	1/38 (3)	1/40 (2)	<4	5
A(H3N2)	67	30	1/27 (4)	2/23 (9)	3/27 (11)	<4	12
B	67	42	0/39	1/35 (3)	1/38 (3)	<4	5

\*A seronegative result was defined as an antibody titer of 1:4 or less.

†An antibody response was defined as an increase in the antibody titer by a factor of four or more.

‡Postvaccination serum was drawn before the second dose of vaccine or on day 35 to day 49 among children in the one-dose cohort.

§This group included subjects who were seronegative (titer, ≤1:4) after the first dose and who had serum samples available for testing after the second dose.



**Figure 1.** Relative Risk of Febrile Disease Regardless of Influenza Culture Results among the Vaccinated Children as Compared with the Placebo Recipients (Top Panel) and the Occurrence of Influenza A and B Infections, as Indicated by Viral Isolation, among the Study Subjects and the General Population at the Study Sites (Bottom Panel) during the 1996–1997 Influenza Season.

enza A(H1N1), and 96 percent had antibodies to each of the other vaccine subtypes.

#### Efficacy

During the interval between vaccination and the end of the influenza outbreaks at the study sites (April 1997), 3009 illnesses among the study subjects were assessed and samples were cultured for influenza virus. The isolation of influenza A or B among the study population paralleled that in the community in general (Fig. 1). Seventy-one subjects had influenza A(H3N2) infections as indicated by viral isolation, with the peak occurrence in the week of December 29, 1996. Forty-four subjects had influenza B, with the peak occurrence among study subjects in the week of February 16, 1997. No infections with wild-type influenza A(H1N1) were identified in either the study subjects or the communities in general during the 1996–1997 influenza season.

Vaccine significantly reduced the occurrence of

culture-confirmed influenza in the study population (Table 4). Among the 1070 children who received vaccine, 14 had culture-confirmed influenza, and among the 532 children who received placebo, 95 had one or more influenza infections. Among the vaccinated children, none had influenza A(H3N2) followed by influenza B, but among the controls, 6 children had two distinct culture-positive episodes of influenza, for a total of 101 illnesses among 95 controls. The vaccine was effective when given in either one or two doses, and it also prevented infection with the two viral subtypes causing disease during this epidemic season, influenza A(H3N2) and influenza B (Table 4).

Among the few children in the vaccine group who had influenza, the spectrum of illness was milder than that in the control group. Only 8 of 14 cases (57 percent) were febrile, and there was only 1 case of otitis media. In contrast, 80 of the 95 children (84 percent,  $P < 0.05$ ) in the placebo group with

**TABLE 4.** EFFICACY OF ONE OR TWO DOSES OF LIVE ATTENUATED, COLD-ADAPTED INFLUENZAVIRUS VACCINE FOR THE PREVENTION OF CULTURE-CONFIRMED INFLUENZA.\*

INFLUENZA TYPE	SUBJECTS ASSIGNED TO ONE DOSE			SUBJECTS ASSIGNED TO TWO DOSES WHO RECEIVED TWO DOSES†			ALL STUDY SUBJECTS‡		
	CASES OF INFLUENZA		EFFICACY (95% CI)	CASES OF INFLUENZA		EFFICACY (95% CI)	CASES OF INFLUENZA		EFFICACY (95% CI)
	Vaccine Group (N=189)	Placebo Group (N=99)		Vaccine Group (N=849)	Placebo Group (N=410)§		Vaccine Group (N=1070)	Placebo Group (N=532)§	
A(H3N2)	2	8	87 (47-97)	4	49	96 (90-99)	7	64	95 (88-97)
B	1	6	91 (46-99)	6	31	91 (78-96)	7	37	91 (79-96)
Any type	3	14	89 (65-96)	10	74	94 (88-97)	14	95	93 (88-96)

\*CI denotes confidence interval.

†This analysis excluded 55 subjects who were randomly assigned to receive two doses of vaccine or placebo. Forty-two received only one dose (see the Methods section), five had received an influenza virus vaccine outside the study (protocol violations), and eight were infected by influenza virus before receiving the second dose.

‡The efficacy calculation includes all children assigned to receive one dose, all children assigned to receive two doses who received two doses, and all other children assigned to two doses who had wild-type influenza before the second dose or for some reason did not receive the second dose. Among the children who did not receive the second dose there was one additional case of influenza in the vaccine recipients and seven additional cases in the placebo recipients.

§Six children had two illnesses with influenza A(H3N2) and influenza B isolated, and all six were in the two-dose cohort. These children are counted once in the calculation of any type of influenza.

culture-positive influenza had fever, and 20 had associated otitis media.

Among the 3009 illnesses for which viral cultures were performed, regardless of culture results, febrile disease was less common in the vaccinated group during the peak of the influenza A(H3N2) outbreak: the week of December 15, 1996 (Fig. 1) (relative risk, 0.5; unadjusted  $P < 0.01$ ). Overall, there were 21 percent fewer febrile illnesses (95 percent confidence interval, 11 to 30 percent; 0.71 per vaccine recipient, as compared with 0.90 per control subject;  $P < 0.001$ ) among the vaccine recipients during the interval between the first dose of vaccine and April 1997. Furthermore, the incidence of febrile otitis media was 30 percent lower among the vaccine recipients (95 percent confidence interval, 18 to 45 percent; 0.14 case of febrile otitis media per vaccine recipient, as compared with 0.20 case per control subject;  $P < 0.001$ ) during this interval.

## DISCUSSION

The placebo recipients in this study had an 18 percent rate of culture-positive influenza, and more than 80 percent of these illnesses were accompanied by fever. This common infection of childhood was also a frequent cause of otitis media, which developed in over 20 percent of placebo recipients who had culture-positive influenza. These data confirm that preventing influenza among children is beneficial. The live attenuated influenza virus vaccine used in this study was administered as a nasal spray and was readily accepted by the children. The vaccine was well tolerated and was not associated with serious adverse events. Some subjects had rhinorrhea or

nasal congestion, low-grade fever, or decreased activity after the first dose but not after the second dose, suggesting that these symptoms were caused by the replication of the viruses in the vaccine. However, we thought that the small increase in the risk of rhinorrhea or nasal congestion (relative risk, 1.5 on day 2 after vaccination) and infrequent low-grade fever (frequency, 6.5 percent on day 2) represented an acceptable level of mild adverse events.

Because several previous studies reported that more than one dose of vaccine was needed to induce serum antibodies to all three viruses in the vaccine in the majority of seronegative children, the efficacy of two doses of vaccine was the primary end point of this study. The second dose was important in that it increased antibody levels to influenza A(H1N1) and, to a much lesser degree, to the other subtypes in the vaccine. The presence of serum antibody, however, is not necessarily the best or the only correlate of protection against influenza after receipt of this live attenuated vaccine. As expected, older children had more preexisting antibody to influenza viruses, particularly influenza A(H3N2), and the presence of these antibodies reflects previous natural infection with antigenically related viruses. After vaccination, antibody responses to the vaccine were significantly more common among the children who were initially seronegative to influenza A(H3N2) (92 percent rate of response after the first dose) than among the children who were initially seropositive (18 percent rate of response).

Nevertheless, vaccine efficacy was equally high for older and younger children; the respective rates of influenza (all types) among children who received pla-

cebo and efficacy in vaccinated children were 17 percent and 86 percent (95 percent confidence interval, 65 to 94 percent) for one-year-olds, 24 percent and 96 percent (95 percent confidence interval, 86 to 99 percent) for two-year-olds, 15 percent and 88 percent (95 percent confidence interval, 68 to 96 percent) for three-year-olds, 17 percent and 100 percent (95 percent confidence interval, 90 to 100 percent) for four-year-olds, and 16 percent and 90 percent (95 percent confidence interval, 69 to 97 percent) for five-year-olds. If the mechanism of efficacy was solely the elicitation of a serum antibody response, there would have been a reduction in efficacy due to a lack of antibody response to the vaccine among older children. Since this was not the case, additional factors, such as stimulation of secretory antibody<sup>20-25</sup> or cellular immunity, must be important mechanisms for the beneficial effect of the live attenuated influenza virus vaccine.

This intranasal influenza virus vaccine seems particularly suited to young children because of its efficacy and ease of administration. Although we did not evaluate the efficacy of inactivated influenza virus vaccine, historical data suggest that the live attenuated vaccine is at least as efficacious, and probably more efficacious, than the inactivated vaccine in children. In the few studies conducted in children that determined point estimates of the efficacy of inactivated vaccine, the values were lower than 90 percent. For example, a recent efficacy study conducted in Japanese children with asthma who were 2 to 14 years of age reported point estimates for inactivated vaccine of 67.5 percent for influenza A(H3N2) and of 43.7 percent for influenza B.<sup>26</sup>

Although bacteria are commonly implicated in the pathogenesis of otitis media, the initial event is often a viral infection with influenza virus or another respiratory tract virus. Cases of otitis media that are associated with viral infections are less responsive to therapy than cases that are not associated with viral infection.<sup>27-30</sup> The prevention of febrile otitis media associated with influenza was a clear benefit of vaccination in our study, with 30 percent fewer cases of febrile otitis media among vaccine recipients than among placebo recipients. Previous studies have also shown a reduction in the incidence of otitis media during influenza outbreaks among children who have received inactivated influenza virus vaccine<sup>31</sup> or live attenuated, cold-adapted intranasal vaccine.<sup>19</sup>

In addition, antibiotics were used significantly less often in the vaccine group in our study. The receipt of vaccine was associated with a 29 percent reduction in the incidence of any febrile illness with concomitant antibiotic use (95 percent confidence interval, 15 to 39 percent;  $P < 0.001$ ) and a 35 percent reduction in febrile otitis media with concomitant antibiotic use (95 percent confidence interval, 18 to 45 percent;  $P < 0.001$ ). Widespread use of influenza-

virus vaccines in children would significantly reduce the frequency of febrile otitis media and of antibiotic use during outbreaks of influenza A or influenza B.

More than half the children had at least one sibling enrolled in the study. This gave us an opportunity to evaluate the ability of the vaccine to reduce the spread of influenza among children who had received placebo but whose siblings had been given the vaccine. Among the cohort of 182 placebo recipients with one vaccinated sibling, there were 32 culture-positive cases of influenza (rate, 18 percent). This rate was the same as the overall attack rate in the placebo cohort (18 percent). Clearly, having a vaccinated sibling was not protective for children given placebo; contact with persons infected with influenza virus was sufficient to spread the virus to unvaccinated children. Therefore, protection of children will require widespread use of influenza virus vaccines at a young age. The characteristics of the live attenuated, cold-adapted, trivalent vaccine that we evaluated make it suitable for routine use in children. Widespread use of this well-tolerated, safe, convenient, and highly efficacious vaccine would substantially benefit children.

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