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A Dose Response Evaluation of Inactivated Influenza Vaccine Given Intranasally and Intramuscularly to Healthy Young Adults

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Abstract

Epidemic influenza occurs annually throughout the world and is accompanied by excess morbidity and mortality. Increasing the antigen content and topical administration of vaccine are two strategies being explored to improve the immune responses to trivalent inactivated influenza vaccine (TIV). We conducted a randomized, double-blind, placebo-controlled trial to compare the immunogenicity and reactogenicity of intramuscular (IM), intranasal (IN), or combined IM and IN administration of a contemporary U.S. vaccine formulation at escalating dosage levels in young healthy adults. 243 healthy adults between the ages of 18 and 45 years received 15, 30, or 60 mcg of trivalent inactivated influenza vaccine by either IN, IM or both routes, 120 mcg of vaccine IM, or placebo IN and IM. All dosages and routes of vaccine administration were well-tolerated. A bad taste and mild nasal discomfort were more likely to be reported when influenza vaccine was administered IN, while arm tenderness was more common after IM administration. Significant increases in geometric mean serum antibody titers in both HAI and Nt assays were seen in all of the groups receiving influenza vaccine for all test antigens ($P \leq .025$, paired t-test), except for the B HAI antibody titer in the group that received 30 mcg IN ($P = .055$, paired t-test). Postvaccination geometric mean serum antibody titers, the frequency of seroresponses, and the percentage achieving postvaccination serum HAI antibody titers of ≥ 32 were higher following delivery of the study vaccines by an IM route than by the IN route, but significant increases in serum antibody were seen after IN vaccination. Nasal IgA antibody responses were more common when vaccine was administered IN; and, when the IN dosage was increased, the primary benefit from IN vaccine over IM vaccine appeared to be greater induction of nasal secretory antibody.

Introduction

Epidemic influenza occurs annually throughout the world and is accompanied by excess morbidity and mortality. Trivalent inactivated influenza virus vaccine (TIV) given intramuscularly (IM) is currently recommended for the prevention of influenza in people considered at high risk for hospitalization and death following influenza virus infection and for those who might transmit virus to them [1]. While TIV has been shown to reduce the

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frequency of influenza illness as well as the severity and frequency of complications [2], many people fail to develop protective antibody responses following immunization [3,4]. Alternative approaches to the immunoprophylaxis of influenza are needed to enhance protection of susceptible individuals.

Increasing the antigen content and topical administration of vaccine are two strategies that we are exploring to improve immune responses to TIV. Several studies have shown dose-related increases in serum antibody responses with IM dosages above the currently recommended level of 15 µg of the influenza virus hemagglutinin (HA) per vaccine strain [3,5-10]. Topical intranasal (IN) administration of inactivated influenza vaccine has been shown to enhance local (nasal) antibody responses over those from inactivated vaccine given IM [11-15]. Combined administration of topical IN and IM inactivated influenza vaccine has also been explored, but dosage ranging studies have not been reported [15,16].

The purpose of this study was to evaluate the immunogenicity and reactogenicity of IM, IN, or combined administration of a contemporary U.S. influenza vaccine formulation at escalating dosages in young healthy adults.

Materials and Methods

Subjects

Healthy adults between the ages of 18 and 45 years were recruited to participate in this study. Eligible subjects did not have acute or chronic illnesses that might interfere with vaccine immunogenicity or reactogenicity, had no history of allergy to vaccine components or history of severe reactions to influenza vaccine and had not received an influenza vaccine in the preceding three years. The protocol was reviewed and approved by the Baylor College of Medicine Institutional Review Board.

Vaccines

The vaccines used in this study were trivalent inactivated influenza virus vaccines manufactured by Aventis Pasteur for the 2001-2002 season and included the following influenza virus strains: A/New Caldeonia/20/99 (H1N1); A/Panama/2007/99 (H3N2); and B/Victoria/504/2000. Three concentrations of vaccine were used: (1) 15-mcg/0.5-ml/strain (Lot U0714A); (2) 30-mcg/0.5-ml/strain (Lot D02275); and (3) 60-mcg/0.5-ml/strain (Lot D02276). Normal saline for injection was used as IN and IM placebo.

Study Design

The study was as a randomized, double-blind, placebo-controlled trial conducted during the fall of 2002 prior to the annual influenza epidemic. After giving informed consent, eligible subjects were randomized to one of 12 study groups (Table 1). Vaccine or placebo was administered by IM injection into the deltoid muscle of a 0.5 ml volume (except groups 10 and 12 who received a 1.0 ml volume) and by IN inoculation as drops into the nose (0.25 ml per nostril). Subjects kept a daily diary of inoculation site and systemic symptoms that occurred during the week following vaccination. Subjects were also evaluated in the clinic 20 minutes after vaccination and at day 2 after vaccination for local and systemic reactogenicity. Symptoms were graded as absent, mild (noticeable but with no activity impairment), moderate (sufficient to interfere with usual activities), or severe (incapacitating or unable to perform usual activities). Local signs of redness and swelling were graded on size (0 = <5mm; 1 = 5-49 mm; 2 = 50-99 mm; 3 = ≥100 mm), not severity. Serum and nasal wash (10 ml) samples were collected prior to vaccination and 4 weeks later for antibody tests. A final phone call was made 6 months after enrollment to identify and document the occurrence of any serious adverse events (SAEs).

Serology

Serum hemagglutination inhibition (HAI) and neutralizing (Nt) antibody assays were performed using previously described methods [17]. Viral antigens used in HAI and Nt assays were the vaccine antigens, with the exception that A/Moscow/10/99 (H3N2), an A/Panama-like virus, was used for A/H3N2 Nt tests.

Total and virus HA-specific nasal wash IgG and IgA antibody levels were measured by ELISA for the influenza A virus strains using previously described methods [6]. Purified HAs derived from influenza A/New Caledonia and A/Panama were used as assay antigens. Nasal wash samples were homogenized, clarified by centrifugation and treated with DNase. Pairs of nasal wash samples, pre- and post-vaccine, were established as evaluable for IgA and IgG if each contained at least 3000 ng total IgA or 300 ng total IgG per 0.1 ml of sample, respectively, and the antibody concentrations for each pair were less than ten-fold different from each other. Total and virus HA-specific IgA and IgG quantities were determined using a nasal secretion IgA and serum IgG reference standard [6], and virus-specific antibody was expressed as a percentage of total IgA or IgG in the sample. A four-fold or greater increase in the percentage of virus-specific antibody was shown to be significant.

Statistical Methods

The primary reactogenicity endpoints were the frequency and severity of solicited local and systemic adverse events. The primary immunogenicity endpoints were the geometric mean titer (GMT) of serum HAI antibody against each of the 3 vaccine antigens one month after immunization and the frequency of nasal wash ELISA antibody responses to the influenza A virus HAs present in the vaccine. Secondary immunogenicity endpoints included the proportion of subjects in each group that achieved a serum HAI antibody titer of ≥ 32 after vaccination and that had significant seroresponses (defined as increases in titer between pre- and postimmunization serum samples of 4-fold or greater) in HAI and Nt assays.

Differences in dichotomous variables were compared by chi-square or Fisher's exact tests and continuous variables were compared using paired t tests and analysis of variance. Linear least squares regression analyses of postimmunization titers were used to evaluate dose response, controlling for preimmunization antibody titers and gender [3]. Statistical analyses were performed using SPSS version 14.0.2 (SPSS, Inc., Chicago, IL).

Results

Two hundred forty three subjects were enrolled into the study. The mean age was 28.9 years (95% CI, 28.1-29.7) and 56% of subjects were women. The age and gender distributions were similar for all study groups. All subjects completed the 4-week follow-up, but 15 persons could not be contacted for the 6-month survey for SAEs.

Reactogenicity

Data on reactogenicity are provided in Table 1. All dosages and routes of vaccine administration were well-tolerated. A transient bad taste (22/121 vs 2/122, $X^2=16.9$, $P<.001$) and mild nasal discomfort (5/121 vs 0/122, $P=.03$, Fisher's exact test) were more likely to be reported when influenza vaccine was administered IN. The bad taste was graded as moderate in two subjects and mild in the rest. Arm tenderness was more common after IM influenza vaccine administration (120/140 vs 15/103, $X^2=118$, $P<.001$) and for higher dosages ($P=.02$, X^2 for linear trend). Other injection site symptoms (erythema and swelling), malaise (49/140 vs 22/31, $X^2=4.7$, $P=.03$) and body aches (31/140 vs 9/103, $X^2=6.8$, $P<.01$) were also more common when vaccine was administered IM. Complaints of severe symptoms of any type were uncommon and were not dosage-related. No vaccine-associated SAEs occurred.

Serum antibody results

Baseline geometric mean serum antibody levels (GMTs) were similar for all groups as measured by HAI and Nt (Tables 3 and 4, respectively). Significant increases in GMTs were seen in all of the groups receiving influenza vaccine and in both HAI and Nt assays for all test antigens ($P \leq .025$, paired t-test) except for the B HAI antibody titer in the group that received 30 mcg IN ($P = .055$, paired t-test). GMTs were higher following delivery of the study vaccines by an IM route than by the IN route. Similarly, the frequencies of seroresponses (Tables 2 and 3) and the percentages achieving post-vaccination serum HAI antibody titers of ≥ 32 (Figure 1) were greater in groups receiving study vaccine by the IM route.

Seroresponses in subjects receiving vaccine only by the IN route occurred most frequently to the H1 test antigen (45-48% and 60-70% by HAI and Nt, respectively) and least frequently to the B test antigen (5-15% and 10-25% by HAI and Nt, respectively, for IN only vaccine groups). Seroresponse frequencies for each test antigen were similar following IM or IM plus IN vaccination (Tables 2 and 3). Subjects with lower prevaccination serum HAI antibody titers ($\text{HAI} < 8$) to the H3 and B test antigens were significantly more likely to have a seroresponse than were subjects with higher prevaccination titers; HAI seroresponses to the H1 antigen, although more frequent in people with low prevaccination serum antibody titers, were not significantly different from those seen in person with higher baseline antibody titers (100/137 vs. 40/64, $X^2 = 1.8$, $P = .18$). Subjects with prevaccination serum Nt antibody titers in the lower half of the study population to each test antigen ($\text{H1 Nt} \leq 8$; $\text{H3 Nt} \leq 24$; $\text{B Nt} \leq 24$) were also significantly more likely to have a seroresponse to the test antigen than were subjects with higher prevaccination titers. Similar results were observed when seroresponse frequencies were analyzed by route of administration; people with lower prevaccination serum antibody titers were more like to have a seroresponse when given vaccine by the IN route or by an IM (+/- IN) route than were those with higher prevaccination titers (except for H1 test antigen measured by HAI).

In linear regression models (not shown), a significant dose-response effect on postvaccination serum antibody titers for each test antigen was seen for IM doses, when controlling for IN dosage and prevaccination titer. Prevaccination serum antibody titer also had a significant positive effect on postvaccination titer for each test antigen, when controlling for dosage and route of administration. However, no dose-response effect on postvaccination serum antibody titers was seen for any of the test antigens based on IN dosage or gender, when controlling for IM dosage and prevaccination titer.

Nasal wash antibody results

The majority of the nasal wash samples collected during the study contained inadequate quantities of IgA (< 3000 ng/0.1 ml) or IgG (< 300 ng/ 0.1 ml) for further analysis. For the adequate pairs of nasal wash samples, more responses (≥ 4 -fold increase in % specific antibody) were seen to the A/New Caledonia (H1N1) hemagglutinin than to the A/Panama (H3N2) hemagglutinin (Table 4). Although the overall numbers of adequate nasal wash specimens were low, subjects receiving the highest dosage of IN vaccine had a local IgA antibody response to at least one of the immunizing HA antigens more frequently than those who received vaccine only by the IM route (40% vs. 11%, $P = .046$, Fisher's exact test). All but one of the subjects with an increase in the % specific IgA antibody in nasal secretions to the test HA also had a four-fold or greater increase in the level of serum neutralizing antibody to the virus; the single exception was a subject with a pre-vaccination serum Nt antibody titer of 128 to A/New Caledonia.

Discussion

A potential strategy for immunizing against influenza infection and disease is a combined approach using IM and IN immunization. In this study we demonstrated that a split product TIV given in varying IM and IN dosages separately and combined was safe and induced both serum and mucosal antibody responses in healthy adults. For the IN vaccine groups, only mild nasal discomfort and a bad taste were seen more frequently than in recipients of IM vaccine alone. Serum antibody response frequencies and postvaccination GMTs were lower following IN-only vaccination than if vaccine was given IM, but mucosal IgA antibody response frequencies were more frequent in IN vaccine recipients and increased with increasing IN vaccine dosages. While IN vaccination increased antibody responses in secretions, adding IN to an IM dosage did not increase the frequency of either serum HAI antibody responses or of those achieving a post-vaccination serum HAI titer of 32 or greater. Overall, the highest dosage of IM vaccine with or without IN vaccine gave the highest HAI and Nt serum antibody responses, suggesting the advantage for IN vaccine is restricted to induction of nasal secretion antibodies.

Administration of inactivated influenza vaccine by the IN route was evaluated extensively during the 1960s [11-13,16]. These studies indicated the use of the IN route would enhance IgA antibody responses in respiratory secretions. Greenbaum and colleagues recently evaluated an inactivated, egg-grown, whole virus vaccine delivered in droplet form as a 20-mcg dosage in a series of studies [14,18,19]. Two- to three-fold increases in serum HAI antibody GMTs were observed following vaccination, and delivery of a second dose or doubling to the dose of vaccine did not appreciably increase the GMTs or seroresponse frequencies [18,19]. These results are similar in magnitude to those observed in the current trial. Nasal antibody immune responses were also observed more frequently in elderly subjects with the inactivated whole virus vaccine compared to an inactivated split product vaccine delivered intramuscularly [14].

Treanor et al. evaluated a monovalent A/ Beijing/262/95 (H1N1) split product vaccine in combination with a proteosome adjuvant delivered as a topical spray in a two-dose regimen [20]. Two- to three-fold increases in serum HAI antibody GMTs were seen in subjects receiving the adjuvanted vaccine, although seroresponses were also seen when adjuvant was not used. The number of subjects evaluated in Treanor's study was too small to determine the impact of the adjuvant on serum immune responses, although the magnitudes of change in GMT and seroresponse frequencies were greater in persons receiving adjuvanted vaccine. Mucosal IgA antibody response frequencies were 35-38% after a single vaccine dose and did not increase with use of the adjuvant but increased to as high as 66% after two doses of adjuvanted vaccine. Langley et al made similar observations in their evaluation of the same adjuvant combined with a split product, trivalent influenza vaccine in healthy adults [21]. Boyce et al. [22] observed seroresponses after a single intranasal dose of trivalent vaccine in 25-50% of subjects whose initial serum HAI antibody level was <40 to the test antigen.

Previous studies have demonstrated that hemagglutinin-specific, mucosal IgA antibodies correlate with protection against influenza virus infection [23-25]. As demonstrated in the current study and in earlier studies [11-13,16,18,19], the delivery of inactivated virus antigen by the topical route is better than by the IM route for induction of local immune responses, and can also induce systemic immune responses, without the use of an adjuvant. Furthermore, it appears possible to enhance the local responses by increasing the IN dosage. In conclusion, increased dosages of IM vaccine alone led to increased serum antibody responses. IN administration of vaccine led to inferior serum antibody responses but to improved nasal IgA responses compared to those achieved by IM administration alone. Thus, the primary benefit from IN vaccine over IM vaccine appears to be a greater induction of nasal secretory antibody.

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Figure 1.

Frequency, by vaccine group, of subjects with postvaccination serum HAI antibody titer ≥ 32 for H1 (A), H3 (B) and B (C) test antigens.

Reactogenicity in the Week Following Vaccination with Different Dosages of Trivalent Inactivated Influenza Vaccine

Table 1

IM dose	IN dose	Number per group	Number (number severe) with:												
			Fever (≥100 F)	Runny Nose	Stuffy Nose	Scratchy Throat	Bad Taste in Mouth	Nasal Discomfort	Arm Pain	Arm Tenderness	Arm Erythema	Arm Swelling	Headache	Malaise	Body aches
0	0	42	1	9	6	9	0	0	2	6	7	3	16	9	4
0	15	21	0	6	7	5	3	0	1	1	1	0	8	3	2
0	30	20	0	4	6	5	5	1	2	5	2	1	8	6 (1)	2 (1)
0	60	20	1	6 (1)	10 (1)	6 (1)	5	0	2	3	6	3	8 (1)	4 (1)	1 (1)
15	0	21	0	4	4	4	0	0	5	14	6	3	6 (1)	7 (1)	5
30	0	19	0	8	9	5	2	0	9	16 (1)	6	5	7 (1)	8 (1)	8
60	0	20	1	8	5	4	0	0	10	20	9	8	4	9	5
120	0	20	1	7	6	5	0	0	10	18	7	8	8	7 (1)	4 (1)
15	15	20	0	4	7	5	2	1	3	16	7	3	5	7	3
30	30	19	0	4	5	3	3	1	6	15	3	3	4	2	0
60	60	21	0	8 (1)	8	6	4	2	13	21	9	5	8 (1)	9 (1)	6 (1)

Table 2
Serum HAI Antibody Responses Following Vaccination with Trivalent Inactivated Influenza Vaccine

IM dose	IN dose	Number per group	H1N1 Prevacc GMT* (95%CI)	H1N1 Postvacc GMT* (95%CI)	H1N1 Fold Rise + (95%CI)	% with ≥ 4- fold rise	H3N2 Prevacc GMT* (95%CI)	H3N2 Postvacc GMT* (95%CI)	H3N2 Fold Rise + (95%CI)	% with ≥ 4- fold rise	B Prevacc GMT* (95%CI)	B Postvacc GMT* (95%CI)	B Fold Rise + (95% CI)	% with ≥ 4- fold rise
0	0	42	2.5 (2.0-3.1)	2.7 (2.1-3.2)	1.1 (1.0-1.2)	0	3.4 (2.9-3.9)	3.7 (3.2-4.2)	1.2 (1.1-1.4)	5	2.2 (1.9-2.5)	2.3 (2.0-2.6)	1.0 (0.9-1.2)	5
0	15	21	2.4 (1.6-3.1)	4.0 (3.2-4.8)	3.1 (1.6-5.9)	48	3.3 (2.6-4.0)	4.3 (3.7-5.0)	2.1 (1.4-3.1)	29	2.8 (2.3-3.3)	3.1 (2.7-3.6)	1.3 (0.9-1.7)	14
0	30	20	2.2 (1.5-2.8)	3.6 (2.6-4.5)	2.6 (1.8-3.8)	45	2.9 (2.3-3.4)	3.6 (3.1-4.0)	1.6 (1.3-2.1)	10	2.7 (2.1-3.2)	3.0 (2.3-3.6)	1.2 (1.0-1.5)	5
0	60	20	1.7 (1.2-2.1)	3.6 (2.8-4.4)	3.9 (2.2-6.8)	45	2.3 (1.7-2.8)	4.5 (4.0-5.0)	4.8 (2.8-8.0)	55	2.1 (1.6-2.6)	2.6 (2.1-3.1)	1.4 (1.1-1.8)	15
15	0	21	2.9 (2.2-3.6)	5.3 (4.2-6.4)	5.2 (2.5-11)	52	3.6 (2.9-4.3)	5.6 (4.7-6.4)	3.9 (2.1-7.2)	57	2.6 (2.1-3.2)	4.3 (3.6-4.9)	3.2 (1.9-5.3)	62
30	0	19	2.5 (1.6-3.3)	5.2 (4.1-6.2)	6.4 (3.6-12)	79	3.3 (2.6-4.1)	5.1 (4.6-5.6)	3.5 (2.1-5.7)	58	2.3 (1.8-2.7)	4.6 (3.9-5.4)	5.2 (3.2-8.4)	63
60	0	20	2.0 (1.4-2.5)	5.7 (4.5-6.9)	14 (6.7-27)	75	3.2 (2.8-3.6)	6.3 (5.6-7.0)	8.6 (5.3-14)	80	2.2 (1.7-2.6)	5.3 (4.6-6.0)	8.9 (4.9-16)	75
120	0	20	2.0 (1.2-2.7)	5.0 (3.8-6.2)	8.3 (4.2-16)	75	3.4 (2.6-4.2)	6.1 (5.6-6.5)	6.3 (4.2-9.5)	80	2.2 (1.6-2.8)	4.4 (3.6-5.2)	4.6 (2.8-7.4)	65
15	15	20	2.5 (1.5-3.5)	5.0 (4.0-5.9)	5.5 (3.0-10)	75	3.1 (2.5-3.6)	5.1 (4.4-5.8)	4.1 (2.4-7.0)	60	2.5 (2.0-2.9)	4.9 (4.3-5.5)	5.5 (3.1-9.7)	75
30	30	19	2.4 (1.4-3.5)	5.5 (4.5-6.5)	8.3 (5.0-14)	84	3.6 (2.7-4.6)	6.3 (5.7-7.0)	6.4 (3.3-13)	63	2.6 (2.1-3.2)	4.6 (4.0-5.3)	4.0 (2.3-6.8)	58
60	60	21	2.4 (1.7-3.2)	5.8 (5.0-6.7)	10 (6.5-17)	81	3.0 (2.1-3.8)	5.6 (5.1-6.1)	6.1 (3.4-11)	76	2.2 (1.6-2.8)	5.1 (4.4-5.8)	7.7 (4.2-14)	71

* log2

+ Fold-rise = ratio of arithmetic values of postvaccination to prevaccination serum antibody titers

Table 3
Serum Neutralizing Antibody Responses Following Vaccination with Trivalent Inactivated Influenza Vaccine

IM dose	IN dose	Number per group	H1N1 Prevacc GMT* (95% CI)	H1N1 Postvacc GMT* (95% CI)	H1N1 Fold Rise+ (95% CI)	% with ≥ 4- fold rise	H3N2 Prevacc GMT* (95% CI)	H3N2 Postvacc GMT* (95% CI)	H3N2 Fold Rise+ (95% CI)	% with ≥ 4- fold rise	B Prevacc GMT* (95% CI)	B Postvacc GMT* (95% CI)	B Fold Rise + (95% CI)	% with ≥ 4- fold rise
0	0	42	4.1 (3.3-4.9)	4.1 (3.3-4.9)	1.0 (0.9-1.2)	0	5.3 (4.6-5.9)	5.4 (4.8-6.1)	1.1 (0.8-1.5)	5	4.5 (3.7-5.2)	4.4 (3.7-5.1)	1.0 (0.8-1.1)	0
0	15	21	4.3 (3.2-5.4)	6.8 (5.6-8.0)	5.5 (2.8-11)	62	4.8 (3.7-6.0)	6.6 (5.5-7.6)	3.4 (2.2-5.3)	48	5.5 (4.8-6.1)	6.1 (5.6-6.7)	1.6 (1.1-2.4)	14
0	30	20	3.2 (2.1-4.3)	5.6 (4.4-6.7)	5.1 (2.8-9.3)	60	4.3 (3.4-5.2)	5.4 (4.6-6.2)	2.1 (1.4-3.1)	25	4.5 (3.5-5.5)	5.1 (4.1-6.1)	1.5 (1.1-2.1)	10
0	60	20	3.2 (2.2-4.3)	7.0 (5.9-8.1)	14 (5.4-33)	70	3.6 (2.7-4.5)	6.0 (5.1-6.8)	5.1 (2.9-8.9)	55	4.0 (2.9-5.1)	5.2 (4.3-6.1)	2.3 (1.2-4.3)	25
15	0	21	4.3 (3.2-5.4)	8.2 (7.1-9.3)	15 (6.7-33)	81	4.5 (3.4-5.6)	7.2 (6.1-8.3)	6.3 (3.4-12)	62	4.8 (3.6-5.8)	8.1 (7.4-8.7)	9.9 (4.8-21)	76
30	0	19	3.8 (2.5-5.2)	8.4 (7.1-9.8)	24 (7.9-75)	84	5.2 (4.1-6.2)	7.8 (7.1-8.4)	6.1 (3.4-11)	68	4.2 (3.3-5.1)	7.8 (6.5-9.2)	12 (5.5-28)	74
	0	20	2.9 (1.8-3.9)	8.4 (7.0-9.8)	48 (18-125)	95	4.3 (3.4-5.2)	7.6 (6.8-8.3)	9.5 (5.3-17)	80	3.3 (2.5-4.2)	8.6 (7.7-9.5)	39 (18-84)	95
120	0	20	2.6 (1.6-3.5)	8.7 (7.5-9.9)	72 (36-147)	95	4.2 (3.3-5.1)	8.0 (7.4-8.7)	14 (8.8-23)	95	3.8 (2.8-4.9)	8.3 (7.2-9.3)	22 (11-44)	85
15	15	20	3.9 (2.6-5.3)	8.8 (7.8-9.7)	28 (14-60)	85	4.8 (4.0-5.7)	7.7 (6.9-8.4)	7.1 (4.1-12)	80	4.8 (3.9-5.6)	8.6 (8.0-9.1)	14 (6.4-30)	70
30	30	19	4.4 (2.9-5.8)	8.7 (7.7-9.7)	20 (9.3-44)	79	4.4 (3.2-5.6)	7.7 (6.7-8.8)	10 (4.8-21)	68	5.4 (4.4-6.4)	8.5 (7.8-9.2)	8.4 (4.8-15)	74
60	60	21	3.6 (2.5-4.7)	10.0 (9.1-10.9)	83 (41-169)	95	4.8 (3.9-5.6)	7.4 (6.8-7.9)	6.0 (3.6-10)	62	3.8 (2.7-4.8)	8.7 (7.9-9.4)	31 (14-68)	86

* log2

+ Fold-rise = ratio of arithmetic values of postvaccination to prevaccination serum antibody titers

Nasal Secretion Antibody Responses to A/New Caledonia (H1N1) and A/Panama (H3N2) Virus in Relation to Dose of Trivalent Inactivated Influenza Virus Vaccine.

Intranasal Dose	IgA			Number (%) with Rise to A/H1N1 or A/H3N2	IgG			Number (%) with Rise to A/H1N1 or A/H3N2
	Number Evaluable	Number (%) with Rise to A/H1N1	Number (%) with Rise to A/H3N2		Number Evaluable	Number (%) with Rise to A/H1N1	Number (%) with Rise to A/H3N2	
0	15	0 (0)	0 (0)	12	0 (0)	0 (0)	0 (0)	
0*	28	2 (7)	1 (4)	21	6 (29)	3 (14)	7 (33)	
15**	16	2 (13)	1 (6)	10	2 (20)	0 (0)	2 (20)	
30**	12	2 (17)	0 (0)	9	2 (22)	1 (11)	2 (22)	
60**	15	5 (33)	3 (20)	10	5 (50)	1 (10)	5 (50)	

* Received 15, 30, 60, or 120 mcg intramuscularly

*** Approximately half received equivalent dose intramuscularly