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High Doses of Purified Influenza A Virus Hemagglutinin Significantly Augment Serum and Nasal Secretion Antibody Responses in Healthy Young Adults

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The reactogenicity and immunogenicity of purified influenza virus hemagglutinin (HA) vaccines administered intramuscularly were evaluated in two placebo-controlled clinical trials. A total of 139 healthy young adults were randomized to receive increasing doses of monovalent influenza A/Taiwan/1/86 (H1N1) virus HA (range, 0 to 405 μg per dose [study 1]). An additional 139 subjects were given increasing doses of a trivalent HA vaccine containing equal amounts of A/H1N1 virus, A/Shanghai/16/89 (H3N2) virus, and influenza B/Yamagata/16/88 virus HA (range, 0 to 135 μg of HA per strain, 0 to 405 μg per dose) or a standard dose of commercial influenza vaccine (study 2). Increasing doses of HA were associated with increasing frequencies of symptoms at the vaccination site early after vaccination, but all doses were well tolerated. Occurrence of systemic symptoms was unrelated to dose. Increasing the dose of HA resulted in increasingly higher postimmunization levels of serum hemagglutination inhibiting and neutralizing antibody levels versus influenza A/H1N1 virus in study 1 ($P < 0.05$); these enhanced responses persisted for up to 6 months. Nasal secretory immunoglobulin A and G antibody responses were assessed 2 weeks after immunization with monovalent H1N1 virus HA; the frequencies of significant responses also increased in a dose-related fashion. Similar increases in serum antibody levels were noted for both A/H1N1 and A/H3N2 viruses in study 2. These data provide a basis for proceeding with the evaluation of high doses of purified HA in the elderly.

Annual immunization with inactivated vaccines is recommended for the prevention of influenza in persons at greatest risk for severe complications and death associated with influenza virus infections (3). The level of protection against influenza virus infection conferred by immunization with inactivated vaccine is strongly correlated with the level of serum antibody produced against circulating influenza virus variants (7). For this reason, the serum antibody level elicited by immunization can be used to predict the efficacy of a particular vaccine.

Commercially available influenza virus vaccines in the United States currently contain 15 μg of hemagglutinin (HA) of each virus per dose. Whole and split virus vaccines containing 15 to 20 μg of HA per strain in each dose elicit levels of serum antibody associated with protection in a majority of susceptible healthy young adults (2, 12). However, this dose of inactivated vaccine often fails to elicit significant antibody responses or levels of antibody that are associated with protection against infections in elderly persons (28).

One approach to improve the efficacy of influenza virus vaccines is to increase the antibody response by increasing the dose of antigen. Previous studies with whole virus or detergent-disrupted subvirion vaccine have indicated that increasing the dose may result in significantly higher antibody levels, but higher doses are associated with increased frequencies of local and/or systemic reactions (1, 19). The objectives of the present studies were to assess the reactogenicity and immunogenicity of increasing doses of highly purified vaccines containing influenza virus HA in healthy young adults prior to their evaluation in elderly subjects.

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MATERIALS AND METHODS

Vaccines. Purified influenza virus HA and commercial trivalent vaccines were generously provided by Connaught Laboratories, Inc., Swiftwater, Pa. The experimental HA vaccines were prepared by bromelain treatment of egg-grown influenza viruses followed by chromatographic purification. Final purified HA migrated as a single band by polyacrylamide gel electrophoresis and failed to elicit antibody to neuraminidase in immunized animals. Two purified HA vaccines were evaluated in separate clinical studies: monovalent A/Taiwan/1/86 (H1N1) virus HA (study 1) and a trivalent vaccine composed of three purified HAs [A/Shanghai/16/89 (H3N2) virus, A/Taiwan/1/86 (H1N1) virus, and B/Yamagata/16/88 virus] (study 2). During study 2, the antigenically identical commercial subvirion vaccine was also administered. Placebo for both studies was sterile saline. Vaccine or placebo was injected as a single 0.5-ml dose into the deltoid muscle.

Clinical procedures. Healthy 18- to 40-year-old adults were recruited at Texas A&M University. Individuals with acute or chronic illness or allergy to eggs and those who were pregnant were excluded. Informed consent was obtained from each participant in accordance with protocols approved by local review boards.

All students were screened for the level of serum antibodies to the test viruses. For study 1, 139 subjects were stratified according to screening antibody (or prestudy) levels versus

influenza A/Taiwan (H1N1) virus into two groups: a high-antibody group (serum HA inhibiting [HAI] titer, ≥1:16) or a low-antibody group (serum HAI titer, ≤1:8, and serum neutralizing antibody, ≤1:12). The neutralizing antibody assay was performed with sera with low HAI antibody levels in order to select the most susceptible subjects. After stratification, subjects in each antibody group were randomized to receive a 15-, 45-, 135-, or 405-μg dose of purified influenza A/Taiwan virus HA (monovalent HA). Volunteers in the high-antibody group were also randomized into an additional placebo arm. Oral temperature was recorded between 6 and 12 h after inoculation, and subjects were examined 1 and 2 days after injection. Blood and nasal wash (NW) specimens for antibody assays were collected before inoculation (preimmunization) and at 2, 4, 14, and 24 weeks after inoculation. For study 1, the average proportions of subjects donating blood samples at 2, 4, 14, and 24 weeks after immunization were 94, 99, 77, and 78%, respectively; for study 2, these proportions were 86, 99, 75, and 80%.

For study 2, 139 subjects were stratified into a high-antibody group [prestudy serum HAI antibody titer, ≥1:16 versus A/Taiwan (H1N1) virus and A/Shanghai (H3N2) virus] or a low-antibody group [serum HAI, ≤1:8, and lowest available neutralizing antibody levels versus A/Taiwan (H1N1) virus, A/Shanghai (H3N2) virus, and/or B/Yamagata virus]. After stratification, subjects were randomized to receive 15, 45 or 135 μg of HA of each strain in the vaccine administered (a total of 405 μg of HA in the highest-dose group) or a standard 0.5-ml dose of the commercial trivalent vaccine. An additional placebo arm was included in the high-antibody group. Monitoring was performed as described for study 1. Attempts to stratify subjects into high- and low-antibody groups on the basis of their prestudy levels of antibody against the three vaccine viruses failed to result in clearly differentiated groups for all viruses; therefore, high- and low- antibody groups were combined into a single group for the purpose of data analysis.

Laboratory procedures. Tests for HAI and neutralizing antibodies on serum samples were performed by using previously described methods (6, 9). For the HAI antibody test, concentrations of reagents were altered and an erythrocyte adsorption step was added to permit a starting dilution of 1:4. Test viruses included A/Taiwan/1/86 (H1N1) virus (studies 1 and 2) and A/Shanghai/16/89 (H3N2) virus and B/Yamagata/16/88 virus (study 2 only). A fourfold or greater rise in serum HAI or neutralizing antibody titer was considered significant.

NW antibody assays after inoculation with monovalent A/Taiwan virus HA or placebo (study 1) were performed by enzyme-linked immunosorbent assay (ELISA) as described previously (14). Purified influenza A/Taiwan/1/86 virus HA was the test antigen. The NW specimens were homogenized, clarified by centrifugation, and tested without concentration. NW pairs suitable for assay contained at least 4.0 μg of immunoglobulin A (IgA) and 0.5 μg of IgG per 0.1 ml in each specimen and total concentrations of IgA or IgG in paired specimens that were not more than 10-fold different from each other, characteristics required to detect and determine the specificity of an antibody increase. The quantities of total and HA-specific antibodies were determined by reference to IgA and IgG standards, and virus-specific antibody was expressed as a percentage of total IgA or IgG in the sample. A fourfold or greater rise in the percentage of HA-specific antibody (IgG or IgA) in nasal secretions represents a significant difference.

Definition of vaccine reactions. Local symptoms (pain and/or tenderness at the injection site) and systemic symptoms (feverishness, malaise, myalgia, headache, and nausea) were graded on a scale of 0 to 3 (0, absent; 1, mild; 2, moderate; 3, severe) on the basis of the volunteers' opinion. A vaccine reaction was considered mild if no fever was reported and the highest sum of systemic or local scores on day 1 or 2 was ≤3, with none being severe. Vaccine reactions were considered moderate if an oral temperature of 100 to 100.9°F (37.8 to 38.27°C) was recorded, or if the highest sum of systemic or local symptom scores was 4 to 6 on day 1 or 2, with none being severe. Oral temperature of ≥101°F (38.3°C), the sum of systemic or local symptom scores on day 1 or 2 of 6 or greater, or any severe local or systemic symptom was defined as a severe vaccine reaction.

Statistical methods. Differences in proportions were compared by using the chi-square test. The dose response of the symptom scores was analyzed via linear least-squares regression, while the dose response for the incidence of fourfold or greater rises in antibody titer was analyzed with the chi-square test for linear trend. The antibody titer levels were analyzed on the $\log_2$ scale. The dose-response analyses were all performed with doses on the $\log_3$ scale, such that doses of 15, 45, 135, and 405 μg were considered equidistant. Analyses were performed with and without the placebo group. For study 1, analyses were performed both separately for the low- and high-prestudy-antibody groups and together for the combined groups. Separate analyses were done for each postinoculation measurement time (2, 4, 14, and 24 weeks). Linear least-squares regression analysis was used to assess the dose response of the antibody titers. Multiple linear regression analysis was used to assess directly whether the dose response was affected by the prestudy antibody titer. Responses to the subvirion vaccine and the low-dose trivalent HA vaccine (each containing 15 μg of HA of three virus strains) in study 2 were compared via Student's *t* test. Antibody levels elicited by immunization with the 15-μg dose of each HA also were compared with those elicited by each of the higher doses by using the two-tailed pooled variance *t* test. All analyses were performed with commercial computer programs (BMDP Statistical Software, Inc., Los Angeles, Calif.).

RESULTS

Reactogenicity. Injection site and systemic symptoms and signs recorded during the first 2 days after immunization with influenza virus vaccines or placebo are summarized in Table 1. A report of mild or moderate pain and/or tenderness at the site of inoculation was common, even with the placebo, and the frequency increased with increasing doses of purified HA in both studies. Erythema and/or induration ≥5 mm in diameter was observed in 3 (12%) of the subjects given the highest dose of monovalent HA. Four to 14% of subjects given trivalent HA developed redness or swelling; no clear dose-related increase in these reactions was apparent. Mean peak injection site symptom scores increased in a dose-related manner during both studies ($P < 0.0005$; linear least-squares regression excluding the placebo group). In contrast, mean peak systemic symptom scores failed to exhibit a relationship to dose in either study. Commercially available subvirion vaccine induced reaction frequencies and severity scores similar to those of the trivalent HA vaccines at all doses and the high-dose monovalent HA vaccine.

Three subjects reported severe symptoms after vaccination with monovalent HA. In two of them (one given 45 μg and one given 135 μg), transient headache reported as severe by the subjects was the only symptom, and in both persons, the headache responded promptly to over-the-counter analgesics. The other severe reaction occurred in a subject given a 45-μg dose of monovalent HA and may have been adventitious; this

TABLE 1. Clinical responses during the 2 days after immunization with purified influenza virus HA vaccines

Vaccine and dose	No. studied	% With injection site reaction:		Mean symptom score	
		Pain or tenderness	Redness or swelling ≥5 mm in diam.	Injection site	Systemic
Monovalent HA (study 1)[a]					
0 μg	24	42	0	0.5	0.1
15 μg	30	33	0	0.4	0.2
45 μg	29	55	0	0.6	0.7
135 μg	30	63	0	0.9	0.4
405 μg	25	92	12	1.7	0.2
Trivalent HA (study 2)[b]					
0 μg	29	52	0	0.6	0.2
15 μg/strain	27	85	8	1.0	0.3
45 μg/strain	28	96	14	1.3	0.6
135 μg/strain	26	100	4	1.7	0.6
Subvirion vaccine (15 μg of HA/strain [study 2])	29	97	7	1.0	0.5

[a] A/Taiwan (H1N1) virus.
[b] A/Taiwan (H1N1) virus, A/Shanghai (H3N2) virus, and B/Yamagata virus.

reaction was characterized by transient fever of 101°F (38.3°C) and symptoms of an acute upper respiratory illness beginning several hours after vaccination. One subject each also reported severe symptoms after receipt of trivalent HA vaccine containing 45 and 135 μg of HA per strain. In the first case, severe fatigue was reported during the day after inoculation; an area (3 by 3.5 cm) of erythema and induration at the injection site was also observed on this volunteer at the time of examination on day 2. The second subject reported severe local tenderness on the day after inoculation, but tenderness elicited by the examiner was minimal; no medications were required, and the reaction did not interfere with the subject's activity. Thus, despite the dose-related increase in local symptoms and an occasional report of a severe symptom, even the highest doses of purified HA vaccine were well tolerated.

Antibody responses. (i) Monovalent HA (study 1). All subjects with low prestudy levels of antibody versus influenza A/Taiwan (H1N1) virus developed fourfold or greater rises in both HAI and neutralizing serum antibody titers after vaccination, whereas the frequencies of significant antibody responses in subjects with high prestudy levels of serum antibody increased in a dose-related fashion (Table 2). In the latter subjects, fourfold or greater rises in serum HAI antibody titers were elicited in 53 to 100% of subjects given 15- to 405-μg doses of HA, respectively ($P < 0.005$; chi-square test for linear trend), while frequencies of significant neutralizing antibody titer rise ranged between 50% and 93% ($P < 0.007$; chi-square test for linear trend).

Geometric mean serum HAI and neutralizing antibody levels elicited by immunization with the ascending doses of purified monovalent influenza A/Taiwan virus HA (range, 15 to 405 μg) are shown in Table 2. A significant dose response in serum HAI antibody levels was observed 2 and 4 weeks after vaccination ($P < 0.05$; linear least-squares regression of titer on dose) but not at 14 or 24 weeks. However, for serum neutralizing antibody, a significant dose response in mean titers was observed at each time point during the 6-month period of study for both the low- and high-antibody groups ($P < 0.01$; linear least-squares regression of titer on dose).

The effects of vaccine dose and preimmunization antibody levels were analyzed with a multiple regression analysis. The mean titer of antibody elicited directly correlated with the preimmunization level of antibody when controlling for dose ($P \leq 0.03$ for HAI and neutralizing antibody at 2, 4, 14, and 24 weeks). For subjects with preimmunization levels of 16 or less, a 27-fold increase in HA dose induced a 2- to 5-fold increase in serum HAI titer 4 weeks after immunization. Serum neutralizing antibody responses exhibited a similar pattern. Similar dose-response patterns were observed when controlling for preimmunization levels of antibody.

The frequencies of fourfold or greater rise in percentage of HA-specific antibodies between preinoculation and 2 weeks postinoculation in NW samples collected from subjects given ascending doses of monovalent A/Taiwan (H1N1) virus HA are shown in Table 3. A significant increase in response frequencies of both IgA and IgG antibodies with increasing vaccine dose was observed ($P < 0.0001$ for both; chi-square test for linear trend). In general, the proportions of subjects with significant rises in percentage of HA-specific antibody levels were higher in the low-antibody group than in the high-antibody group when controlling for dose. Two immunized subjects developed significant NW antibody responses (one with IgA and IgG rises and one with an IgG rise only) in the absence of a serum antibody response; both were in the group with high prestudy serum antibody levels. Fifteen subjects had detectable serum antibody responses with neither an IgG nor an IgA antibody response in NW (7 in the high-antibody group and 8 in the low-antibody group); 14 of these 15 were given one of the lower doses of vaccine (15 or 45 μg [data not shown]).

(ii) Trivalent HA (study 2). Serum HAI and neutralizing antibody responses against the three vaccine viruses were similar at all postvaccination times for groups given standard subvirion vaccine or the comparable purified HA vaccine containing 15 μg of HA per strain ($P > 0.05$; Student's t test) (Table 2, trivalent HA). Responses that were significantly greater than those of the 15-μg dose of HA are noted in the table.

Turning to individual components of the trivalent vaccine, serum antibody response patterns versus influenza A/Taiwan (H1N1) virus after immunization with ascending doses of purified trivalent HA vaccine (range, 15 to 135 μg per strain per dose) were similar to those observed after immunization with monovalent influenza A/Taiwan (H1N1) virus HA. Significant correlation of the increases in serum HAI antibody levels with increasing doses of vaccine were observed between

TABLE 2. Serum antibody responses after immunization with subvirion influenza virus vaccine or ascending doses of purified influenza virus HA

Study	Vaccine virus	Dose (μg) of HA[a]	HAI antibody response						Neutralizing antibody response					
			Geometric mean titer at indicated wk					% With >4-fold rise	Geometric mean titer at indicated wk					% With >4-fold rise
			0	2	4	14	24		0	2	4	14	24	
Monovalent HA (study 1)	A/Taiwan (H1N1) (low-antibody group)	15 (14)	6	97	97	74	52	100	23	1,448	1,552	724	776	100
		45 (14)	7	169	137	119	97[b]	100	20	2,521	2,353	1,663	1,176	100
		135 (15)	6	147	158	74	64	100	14	2,896	2,195	1,552	891	100
		405 (14)	6	315[b]	208[b]	119	69	100	11	6,208[b]	4,390[b]	2,702[b]	2,353[b]	100
	A/Taiwan (H1N1) (high-antibody group)	15 (15)	42	97	128	84	69	53	549	1,351	1,552	955	1,024	50
		45 (15)	39	274[b]	294[b]	256[b]	169[b]	87	388	4,096[b]	3,822[b]	3,104[b]	3,327[b]	73
		135 (15)	37	223[b]	274[b]	169	111	87	274	3,566[b]	2,896	2,195[b]	2,896[b]	93
		405 (10)	34	315[b]	239	208[b]	128	100	294	5,405[b]	4,705[b]	3,822[b]	3,327[b]	90
Trivalent HA (study 2)	A/Taiwan (H1N1)	Subvirion[c] (29)	20	61	64	59	50	55	133	781	867	714	653	62
		15 (27)	17	64	66	52	43	63	123	1,201	982	855	724	78
		45 (28)	14	121[b]	100[b]	70	49	86	130	2,402[b]	1,808[b]	1,389	969	75
		135 (26)	16	137[b]	108[b]	86[b]	62	73	70	1,499	1,468	1,105	781	81
	A/Shanghai (H3N2)	Subvirion	11	40	34	29	25	62	36	272	204	236	207	66
		15	8	33	25	24	21	63	24	212	208	163	160	78
		45	11	59[b]	49[b]	33	24	68	35	560[b]	465[b]	333	251	93
		135	7	84[b]	56[b]	33	24	85	32	792[b]	505[b]	302	324	92
	B/Yamagata	Subvirion	5	27	23	22	19	66	10	223	202	153	142	83
		15	5	28	27	21	17	70	7	152	124	108	103	78
		45	5	36	28	17	14	86	5	271	179	202	144	89
		135	4	29	22	14	13	88	5	145	104	59	66	100

[a] See Materials and Methods for description. *n*, number of subjects enrolled with at least one postimmunization blood sample.
[b] Significantly greater than the geometric mean titer elicited by the 15-μg dose (pooled-variance two-tailed *t* test).
[c] Commercial subvirion vaccine contained 15 μg of HA per strain per dose.

2 weeks and 14 weeks after inoculation ($P < 0.02$; linear least-squares regression of titer on dose). A significant dose response in serum neutralizing antibody levels was detected only at the 4-week time point when controlling for the preimmunization antibody level ($P = 0.047$; multiple regression analysis).

Significant dose-response relationships at 2 and 4 weeks were observed for both serum HAI and neutralizing antibody responses against influenza A/Shanghai (H3N2) virus after immunization with purified trivalent HA (15 to 135 μg per dose). Two- to threefold increases in geometric mean HAI titers in sera collected 4 weeks after immunization were observed for subjects given up to a ninefold increase in the dose of purified HA when controlling for the preimmunization serum antibody level. Most volunteers with low levels of preimmunization antibody versus A/H3N2 virus developed significant serum antibody responses; the frequencies of rise in those with preimmunization serum HAI antibody levels of 16 or greater were 38% in the group given 15 μg and 60% in those given 135 μg of influenza A/Shanghai virus HA in a trivalent vaccine (data not shown).

A dose-related increase in frequencies of significant serum antibody responses to B/Yamagata virus was also observed after administration of purified trivalent HA vaccines, although this dose response was not reflected in geometric mean serum antibody titers (Table 2, trivalent HA).

TABLE 3. Antibody responses in nasal secretions after intramuscular immunization with purified influenza A/Taiwan (H1N1) virus HA

Dose of HA (μg)	No./total (%) with rise in[a]:			
	Low-antibody group		High-antibody group	
	IgA	IgG	IgA	IgG
0			0/20 (0)	0/20 (0)
15	6/12 (50)	8/12 (67)	3/14 (21)	5/14 (36)
45	8/14 (57)	9/14 (64)	4/13 (31)	9/13 (69)
135	11/13 (85)	13/13 (100)	7/12 (58)	12/12 (100)
405	9/10 (90)	10/10 (100)	4/7 (57)	6/7 (86)

[a] Values reflect numbers of subjects with fourfold or greater rise in percentage of HA-specific antibody as determined with an ELISA.

DISCUSSION

These studies demonstrate that purified influenza virus HA vaccine is well tolerated when given intramuscularly in doses of up to 405 μg of HA. Increasing the dose of purified influenza A/H1N1 and A/H3N2 virus HA resulted in significant increases in serum antibody titers. These enhanced serum antibody responses were associated with enhanced nasal secretory antibody responses in subjects given the monovalent A/H1N1 virus vaccine. The levels of serum antibody achieved after immunization were directly correlated with preimmunization levels. Failure to elicit significant antibody responses to standard doses of HA in subjects with high preexisting levels of serum antibody could be overcome by increasing the dose of vaccine.

A number of factors may influence the frequency and magnitude of serum antibody response after parenteral immunization with influenza virus vaccines, including age, state of

health, prior infection or immunization (priming), type and dose of vaccine, and preexisting antibody level. The effect of dose on serum antibody responses to whole virus and subunit influenza vaccines has been investigated previously. In general, small increments in dose (less than or equal to two- to fourfold) fail to elicit significantly higher levels of serum antibodies (5, 8, 13, 20, 21, 27). Higher increments (10-fold or greater than standard doses) consistently have been shown to enhance serum antibody responses (15, 16, 18, 19, 23), although the slope of the dose-response curve is rather flat. Enhanced protection against naturally occurring influenza virus was observed after administration of higher doses of vaccine to prisoners (15, 25) and members of a retirement community (25).

Although increasing the dose of influenza virus vaccine can improve antibody responses, the administration of high doses of vaccine can be associated with increased frequencies of adverse reactions. Mostow et al. reported a clear relationship between dose of vaccine and the occurrence of local reactions after subcutaneous injection (via jet injector gun) of zonal purified influenza A/Japan/305/57 (H2N2) virus vaccine in the range of 300 to 4,800 chick cell agglutinating (CCA) units per dose (19). The frequency of local reactions was markedly reduced when similar or higher doses of subunit vaccine were given intramuscularly (23). Adverse effects, including fatigue, malaise, and fever, were noted to occur more frequently in a group of soldiers accidently given 10 times the standard dose in a whole virus vaccine containing 210 μg of hemagglutinin than in a control group given the standard dose (18); the frequency of local reactions was not reported.

The effect of increasing the dose of vaccine on nasal secretory antibody responses is less completely characterized than that for serum antibody. Ruben and colleagues measured NW neutralizing antibody responses in young adult volunteers given low (400-CCA-unit) and high (6,400-CCA-unit) doses of tri-(*n*-butyl) phosphate–split influenza A/Aichi/68 (H3N2) virus vaccine intramuscularly (24). The frequency of NW antibody response was 77% in the high-dose group compared with 36% in the low-dose group. Similar results occurred with an influenza A2/Japan/305/57 virus vaccine: 40% of subjects given 300 CCA units intramuscularly developed NW neutralizing antibodies after immunization, compared with 80% of those given 4,800 CCA units (19). The frequencies of NW IgG and IgA antibody responses in the present study exhibited a dose-related pattern similar to those reported previously, despite differences in vaccine type and the methods used for detection of NW antibodies.

Because mucosal antibody can contribute to protection against influenza virus infection, it seems reasonable to suggest that the optimal vaccination strategy for prevention of influenza should elicit both local and systemic immune responses. Several reports have shown that live influenza viruses administered as nose drops are superior to standard doses of inactivated vaccines administered parenterally with regard to their ability to elicit nasal secretory antibody responses (4, 10, 17). In most studies, the frequencies of NW IgA antibody responses were higher after intranasal vaccination with live attenuated influenza virus vaccines than after intramuscular immunization with low doses (15 to 20 μg) of inactivated vaccine (10, 11, 30). Standard doses of inactivated vaccines given intramuscularly typically elicit NW IgG antibody responses in about one-third to one-half of adults (4, 10, 11, 26), although over 90% of subjects with low preinoculation levels of antibody developed NW IgG antibody responses after a standard dose of ether-treated vaccine given intramuscularly in one study (4). The pattern of NW IgG response after intranasal vaccination with live virus is variable. Inactivated vaccine given intranasally elicits more secretory antibody responses than when given intramuscularly, but serum antibody responses in volunteers vaccinated intranasally are significantly lower (29, 30). Our data demonstrate that the frequencies of NW antibody responses (IgG and IgA) after intramuscular immunization can approach those achieved after intranasal vaccination with either live or inactivated vaccine by increasing the dose administered.

Inactivated influenza virus vaccines are currently recommended for groups of high-risk people without regard to their various levels of antibody to vaccine viruses. For persons with intermediate to high levels of preexisting antibody, commercially available vaccines often fail to elicit significant serum and/or nasal secretory antibody responses. Our data suggest that increasing the dose with a purified HA vaccine is a promising approach for the development of improved vaccines for the prevention of influenza and provide a rationale for evaluating the use of high-dose vaccines in the elderly.

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