

ORIGINAL ARTICLE

Serum Antibody Responses after Intradermal Vaccination against Influenza

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ABSTRACT

BACKGROUND

If found to be safe and immunogenic, reduced doses of influenza vaccine given by the intradermal route could increase the number of available doses of vaccine.

METHODS

In an open-label study, we randomly assigned 119 subjects to receive an intradermal injection of trivalent inactivated influenza vaccine, containing 6 μ g of hemagglutinin for each antigen (40 percent of the usual dose), and 119 to receive an intramuscular injection of the standard dose of 15 μ g of hemagglutinin for each antigen. The two groups were subdivided according to age (18 to 60 years and older than 60 years).

RESULTS

Among subjects who were 18 to 60 years of age, serum antibody responses were vigorous and did not differ significantly between the intradermal and intramuscular groups, and all subjects had hemagglutination-inhibition (HAI) titers of at least 1:40. Although the subjects who were older than 60 years of age also had a vigorous antibody response, there was a trend toward a better response in the intramuscular route, but this finding was significant only for antigen to the H3N2 strain. Nevertheless, 100 percent of older subjects in the intramuscular group and 93 percent of such subjects in the intradermal group had an HAI antibody titer to the H3N2 strain of more than 1:40, and 100 percent in each group had a titer of this level for both the H1N1 and B strains. Local pain was significantly more common in the intramuscular group than in the intradermal group among subjects who were 18 to 60 years of age but not among subjects who were over 60 years old. Signs of local inflammation were significantly more common among subjects in the intradermal group than among those in the intramuscular group, in both age groups.

CONCLUSIONS

As compared with an intramuscular injection of full-dose influenza vaccine, an intradermal injection of a reduced dose resulted in similarly vigorous antibody responses among persons 18 to 60 years of age but not among those over the age of 60 years.

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INTRADERMAL ADMINISTRATION OF ANTIGENS is expected to facilitate their exposure to antigen-presenting cells, such as macrophages and dendritic cells, which are present at higher levels in skin than in muscle.¹ Therefore, as compared with intramuscular vaccination, intradermal vaccination may induce similar serum antibody responses with a smaller quantity of antigen. The intradermal route has been evaluated for influenza, rabies, and hepatitis B virus vaccines.²⁻⁵ Brown et al. found that the intradermal administration of one fifth of the standard dose of A/Swine/NJ/76 influenza vaccine produced antibody titers similar to those elicited by the standard intramuscular dose in healthy adults and resulted in fewer systemic reactions.⁶ We evaluated the safety and immunogenicity of the intradermal injection of a candidate influenza vaccine containing 6 µg of hemagglutinin antigen per strain in two groups of adults, one 18 to 60 years of age and the other over 60 years of age. A U.S.-licensed influenza vaccine administered intramuscularly at the standard dose was used as the reference vaccine.

METHODS

SUBJECTS AND STUDY DESIGN

The primary objective of the study was to evaluate the immunogenicity of intradermal vaccination with a candidate influenza vaccine containing 6 µg of hemagglutinin antigen of each strain in the vaccine, as measured by the titer of hemagglutination-inhibition (HAI) antibodies, in order to determine whether it met the guidelines of the European Committee for Proprietary Medicinal Products (CPMP) for the annual relicensure of influenza vaccines. The current CPMP guidelines are summarized as follows: for adults 18 to 60 years old, the seroconversion rate must exceed 40 percent, the seroconversion factor must exceed 2.5, and the seroprotection rate must exceed 70 percent; the respective values for adults over the age of 60 years are more than 30 percent, more than 2.0, and more than 60 percent. The seroconversion rate is the percentage of vaccine recipients who have an increase in serum HAI titers by at least a factor of 4 after vaccination, as compared with titers before vaccination. The seroconversion factor is the fold increase in serum HAI titers after vaccination (the postvaccination antibody titer divided by the prevaccination antibody titer), and the seroprotection rate is the percentage of vaccine recipients with a serum HAI titer

of at least 1:40 after vaccination. To meet the CPMP guidelines, each of the vaccine antigens must meet at least one of the above criteria.

Secondary objectives included a comparison of the immunogenicity and safety of the candidate vaccine administered intradermally at a reduced dose with those of the reference vaccine, a U.S.-licensed influenza vaccine administered intramuscularly at the standard dose. We also evaluated the severity of pain at the administration site just after the injection, using a visual-analogue scale.

A total of 238 subjects were enrolled at two clinical centers: 130 men and women 18 to 60 years of age and 108 men and women over 60 years of age. The subjects were divided into four groups of approximately 60 subjects each. All subjects gave written informed consent, and the study was approved by the institutional review boards at Saint Louis University and the University of Rochester. Subjects were free of obvious health problems before enrollment, as established by a review of their medical history and a clinical examination. Subjects were excluded if they had been taking immunosuppressants or other immune-modifying drugs within two months before vaccination or were pregnant or lactating.

The trial design was developed by investigators at GlaxoSmithKline with advice from the study investigators. Data were gathered by the study investigators and transmitted to the sponsor, which was responsible for maintaining the database and analyzing the data. The data were interpreted jointly by the study investigators and the sponsor. Dr. Belshe, the lead investigator, drafted the manuscript and made decisions about publication. The investigators had full and unfettered access to the data.

STUDY VACCINES AND VACCINE ADMINISTRATION

Each 0.5-ml dose of the U.S.-licensed influenza vaccine used in the study (Fluzone, Aventis Pasteur, lot SSW200101C7) contained 15 µg of hemagglutinin antigen of A/New Caledonia/20/99 (H1N1), 15 µg of hemagglutinin antigen of A/Panama/2007/99 (H3N2), 15 µg of hemagglutinin antigen of B/Victoria 504/2000, 0.05 percent gelatin (as a stabilizer), and 25 µg of mercury as thimerosal preservative. This commercial vaccine was assumed to comply with the specifications given in the manufacturer's summary of product characteristics, was used in full adult doses of 0.5 ml for intramuscular injection, and was supplied in 5-ml multidose vials. The vial was shaken before each dose was with-

drawn. Shaking the vaccine results in an essentially clear and slightly opalescent liquid. The sponsor provided 25-gauge, 25.4-mm needles attached to 1-ml syringes for the intramuscular injections. The vaccine was administered intramuscularly in the region of the deltoid muscle of the nondominant arm.

The vaccine used for intradermal injections (GlaxoSmithKline, lot DFLU50A15) was supplied in prefilled 0.5-ml glass syringes containing 0.1 ml of vaccine. This vaccine, which is not currently licensed for use in the United States, is a trivalent inactivated vaccine prepared at twice the standard concentration, with each dose containing 6 μ g of hemagglutinin antigen of A/New Caledonia/20/99 (H1N1), 6 μ g of hemagglutinin antigen of A/Panama/2007/99 (H3N2), 6 μ g of hemagglutinin antigen of B/Johannesburg 5/99, and less than 0.25 μ g of mercury as thimerosal. The resulting dose of vaccine represented 40 percent of the intramuscular dose. Vaccine was administered intradermally with the use of a tuberculin syringe with a 30-gauge beveled needle that protruded by 1.5 mm from a plastic disk to limit skin penetration, thus ensuring that the vaccine was administered intradermally and not subcutaneously. The syringe was placed perpendicular to the skin of the deltoid region of the nondominant arm and introduced firmly into the skin until the skin came in close contact with the disk limiting penetration. Light pressure was maintained during injection to ensure continuous contact of the disk with the skin and to limit any leakage of the vaccine from the injection site. The injection lasted approximately three to five seconds. Wheal formation confirmed that the intradermal injection had been effective. All subjects were observed for 30 minutes after vaccination.

RANDOMIZATION

The randomization was performed at GlaxoSmithKline Biologicals, in Rixensart, Belgium, with the use of a standard SAS program. A block randomization scheme (1:1 ratio) was used to ensure that balance between the two treatments was maintained. A unique randomization number identified the vaccine dose administered to each subject, as well as the device used. On day 0, subjects, depending on their age, were given the vaccine dose with the lowest number still available at the study center. The vaccine number was also used as a subject identifier for all data collected during the study.

MEASUREMENT OF REACTOGENICITY

Immediately after vaccination, subjects were asked to rate the painfulness of the injection with the use of a nongraduated visual-analogue scale, ranging from 0 mm (no pain) to 100 mm (worst possible pain). Each subject drew a vertical line on the scale, and the distance from zero was taken as the measure of the severity of pain. In addition, subjects were given diary cards to record any of the following immunization reactions that occurred on the day of vaccination or during the six subsequent days: pain, redness, swelling, induration, or ecchymosis at the injection site; fever (a temperature of at least 37.5°C [99.5°F]); headache; malaise; shivering; myalgia; and arthralgia. Information on serious adverse events was collected throughout the trial.

ASSESSMENT OF IMMUNOGENICITY

A serum sample was collected from each subject before vaccination and approximately 21 days (range, 21 to 28) after vaccination. Serum samples were separated into aliquots and stored at a temperature of -20°C or lower until assayed. The standard HAI assay was conducted at GlaxoSmithKline Biologicals (Dresden, Germany) to determine antibody titers against each of the strains of influenza included in the vaccine. The strains of influenza A/H1N1 and influenza A/H3N2 used in the two vaccines were the same, but the influenza B strains were different. The intramuscular vaccine contained B/Victoria 504/2000, whereas the intradermal vaccine included the closely related B/Johannesburg 5/99 strain. Since the serum samples were tested in a blinded fashion (i.e., the laboratory staff did not know which vaccine was administered to the subjects), all samples were tested against both of the influenza B strains as well as the homologous influenza A/H1N1 and influenza A/H3N2 strains.

STATISTICAL ANALYSIS

The sample size was determined on the basis of the number needed to meet annual licensing requirements for influenza vaccine in Europe. In accordance with CPMP criteria, 60 subjects per group were to be recruited in order to have at least 50 subjects with data that could be evaluated. The power to meet the CPMP criteria for all strains was at least 100 percent minus the sum of the beta coefficients for each strain and age group. Specifically, if the true seroconversion rate for strains was at least 55 percent among adults 18 to 60 years of age and at

least 45 percent among those older than 60 years, the study had a statistical power of at least 88 percent to meet the CPMP criteria with the use of the seroconversion rate alone.

Analyses were performed with the use of SAS software, version 6.12. Only descriptive data are presented for immunogenicity and reactogenicity. The geometric mean titer of each strain was calculated with the use of the log-transformed values from all subjects; the geometric mean titer was taken as the antilog of the mean of the transformed values. Ninety-five percent confidence intervals for the ratio of the geometric mean titers were calculated, with intervals that excluded 1 indicating possible differences between groups.

According to the protocol, the safety analysis included all subjects given an injection for whom data were recorded, and the analysis of immunogenicity included all subjects with paired serum samples collected on day 0 (before vaccination) and 21 to 28 days after vaccination.

RESULTS

The first subject was enrolled on October 26, 2001, and the last study visit was on January 10, 2002. A total of 238 subjects were enrolled and randomly assigned to the two treatment groups: 119 to the intramuscular group and 119 to the intradermal group. The subjects were also divided into two age strata: 130 subjects were 18 to 60 years of age, and 108 subjects were over 60 years of age.

All 238 subjects completed both study visits and received the vaccine according to the protocol. No data were available for 1 subject in the intradermal group, leaving 237 subjects eligible for inclusion in the reactogenicity analysis according to the protocol. Data from 123 of the 130 subjects who were 18 to 60 years old and from 102 of the 108 subjects over 60 years of age were eligible for inclusion in the immunogenicity analysis according to the protocol. Eleven of the 13 subjects who were excluded from the serologic analysis were noncompliant with the blood-drawing schedule, and essential data were missing for 2 subjects. The age and sex distribution of the subjects in each age group is given in Table 1.

Among the younger subjects (18 to 60 years of age), there were no significant differences between the intradermal group and the intramuscular group in the geometric mean HAI titers of any of the antigens after vaccination (Table 2); all subjects had titers of at least 1:40 in response to each of the three

Table 1. Demographic Characteristics of the Subjects.

Group	No. of Subjects	Age (yr)	
		Mean \pm SD	Range
18–60 Yr of age			
Intramuscular	69	39.3 \pm 11.3	18–60
Women	45	39.0 \pm 10.6	21–58
Men	24	39.8 \pm 12.8	18–60
Intradermal	61	39.6 \pm 11.6	18–60
Women	37	38.5 \pm 10.1	20–55
Men	24	41.2 \pm 13.6	18–60
All subjects	130	39.4 \pm 11.4	18–60
Women	82	38.8 \pm 10.3	20–58
Men	48	40.5 \pm 13.1	18–60
>60 Yr of age			
Intramuscular	50	69.8 \pm 7.6	61–91
Women	29	70.8 \pm 8.3	61–91
Men	21	68.5 \pm 6.4	62–83
Intradermal	58	68.7 \pm 5.5	61–82
Women	27	69.1 \pm 5.8	61–80
Men	31	68.3 \pm 5.2	61–82
All subjects	108	69.2 \pm 6.5	61–91
Women	56	70.0 \pm 7.2	61–91
Men	52	68.4 \pm 5.7	61–83

strains in the vaccines. Mean fold increases in titers did not differ significantly between the groups; the slightly lower fold increase in the response to the strain B antigens in the intradermal group (2.4 for B/Johannesburg and 2.4 for B/Victoria) and the lower seroconversion rates (25.0 percent and 26.7 percent, respectively) may have been due to the higher antibody titers to influenza B in this group before vaccination (reciprocal HAI titers of 214 and 214, respectively, as compared with the respective values of 140 and 156 in the intramuscular group).

Among the older subjects (over 60 years of age), the postvaccination geometric mean HAI titers were generally lower for all antigens than those in younger subjects, and the titers fell outside the lower boundary of the 95 percent confidence interval of the titer in younger subjects for the H1/New Caledonia and B/Victoria strains in the intramuscular group and for all four strains in the intradermal group (Tables 2 and 3). The intramuscular route resulted in higher geometric mean HAI titers for A/Panama (H3N2) than the intradermal route among the subjects over 60 years old (ratio of the

Table 2. Geometric Mean HAI Antibody Titers before and after Intramuscular or Intradermal Vaccination of Subjects 18 to 60 Years of Age.*

Variable	Intramuscular Group (N=63)	Intradermal Group (N=60)	<i>value (95% CI)</i>	
Geometric mean titer†				
A/New Caledonia (H1N1)				
Before vaccination	93 (69–125)	90 (62–131)		
After vaccination	361 (280–467)	359 (271–476)		
A/Panama (H3N2)				
Before vaccination	75 (54–104)	64 (47–91)		
After vaccination	271 (214–344)	258 (203–329)		
B/Johannesburg				
Before vaccination	140 (111–178)	214 (166–275)		
After vaccination	508 (413–625)	517 (420–636)		
B/Victoria				
Before vaccination	156 (122–198)	214 (161–283)		
After vaccination	522 (425–641)	514 (411–642)		
Seroconversion rate (%)				
A/New Caledonia (H1N1)	42.9 (30.5–56.0)	31.7 (20.3–45.0)		
A/Panama (H3N2)	33.3 (22.0–46.3)	35.0 (23.1–48.4)		
B/Johannesburg	42.9 (30.5–56.0)	25.0 (14.7–37.9)		
B/Victoria	44.4 (31.9–57.5)	26.7 (16.1–39.7)		
Seroconversion factor‡				
A/New Caledonia (H1N1)	3.9 (2.9–5.3)	4.0 (2.6–6.0)		
A/Panama (H3N2)	3.6 (2.6–5.1)	4.0 (2.7–5.7)		
B/Johannesburg	3.6 (2.7–4.8)	2.4 (1.9–3.2)		
B/Victoria	3.4 (2.5–4.5)	2.4 (1.7–3.4)		
Seroprotection rate (%)				
A/New Caledonia (H1N1)				
Before vaccination	77.8	75.0		
After vaccination	100.0	100.0		
A/Panama (H3N2)				
Before vaccination	79.4	73.3		
After vaccination	100.0	100.0		
B/Johannesburg				
Before vaccination	93.7	98.3		
After vaccination	100.0	100.0		
B/Victoria				
Before vaccination	93.7	95.0		
After vaccination	100.0	100.0		

* Values were compared with the use of the following CPMP guidelines: a seroconversion rate of more than 40 percent, a seroconversion factor of more than 2.5, and a rate of seroprotection (defined as a titer of 1:40 or more) of more than 70 percent. Prevacination blood samples were obtained at the time of vaccination, and postvaccination samples were obtained between day 21 and day 28. None of the responses to intradermal vaccination differed significantly from the responses to intramuscular vaccination by Fisher's exact test. CI denotes confidence interval.

† The ratio (and 95 percent confidence interval) of the geometric mean titer after intramuscular vaccination to the titer after intradermal vaccination was 1.0 (0.7 to 1.5) for the A/New Caledonia strain, 1.1 (0.8 to 1.5) for the A/Panama strain, 1.0 (0.7 to 1.3) for the B/Johannesburg strain, and 1.0 (0.8 to 1.4) for the B/Victoria strain.

‡ The seroconversion factor is the ratio of the geometric mean titer before vaccination to the titer after vaccination.

Table 3. Geometric Mean HAI Antibody Titers before and after Intramuscular or Intradermal Vaccination of Subjects over 60 Years of Age.*

Variable	Intramuscular Group (N=46)	Intradermal Group (N=56)	<i>value (95% CI)</i>	
Geometric mean titer†				
A/New Caledonia (H1N1)				
Before vaccination	75 (55–103)	83 (65–105)		
After vaccination	180 (141–232)	155 (125–193)		
A/Panama (H3N2)				
Before vaccination	65 (44–95)	66 (48–92)		
After vaccination	238 (168–338)	136 (100–186)‡		
B/Johannesburg				
Before vaccination	190 (142–255)	165 (130–209)		
After vaccination	416 (326–532)	332 (265–416)		
B/Victoria				
Before vaccination	183 (136–248)	172 (136–218)		
After vaccination	378 (301–474)	293 (235–366)		
Seroconversion rate (%)				
A/New Caledonia (H1N1)	26.1 (14.3–41.1)	17.9 (8.9–30.4)		
A/Panama (H3N2)	39.1 (25.1–54.6)	16.1 (7.6–28.3)§		
B/Johannesburg	26.1 (14.3–41.1)	17.9 (8.9–30.4)		
B/Victoria	23.9 (12.6–38.8)	17.9 (8.9–30.4)		
Seroconversion factor¶				
A/New Caledonia (H1N1)	2.4 (1.8–3.2)	1.9 (1.6–2.2)		
A/Panama (H3N2)	3.7 (2.4–5.7)	2.1 (1.6–2.7)		
B/Johannesburg	2.2 (1.7–2.9)	2.0 (1.6–2.5)		
B/Victoria	2.1 (1.6–2.7)	1.7 (1.4–2.1)		
Seroprotection rate (%)				
A/New Caledonia (H1N1)				
Before vaccination	82.6	89.3		
After vaccination	100.0	100.0		
A/Panama (H3N2)				
Before vaccination	73.9	75.0		
After vaccination	100.0	92.9		
B/Johannesburg				
Before vaccination	93.5	96.4		
After vaccination	100.0	100.0		
B/Victoria				
Before vaccination	95.7	98.2		
After vaccination	100.0	100.0		

* Values were compared with the following CPMP guidelines: a seroconversion rate of more than 30 percent, a seroconversion factor of more than 2.0, and a rate of seroprotection (defined as a titer of 1:40 or more) of more than 60 percent. Pre-vaccination blood samples were obtained at the time of vaccination, and postvaccination samples were obtained between day 21 and day 28. CI denotes confidence interval.

† The ratio (and 95 percent confidence interval) of the geometric mean titer after intramuscular vaccination to the titer after intradermal vaccination was 1.2 (0.8 to 1.6) for the A/New Caledonia strain, 1.8 (1.1 to 2.8) for the A/Panama strain, 1.3 (0.9 to 1.7) for the B/Johannesburg strain, and 1.3 (0.9 to 1.8) for the B/Victoria strain.

‡ P=0.009 by Student's t-test.

§ The odds ratio for seroconversion in the intradermal group as compared with the intramuscular group was 0.30 (95 percent confidence interval, 0.10 to 0.82; P=0.02 by Fisher's exact test).

¶ The seroconversion factor is the ratio of the geometric mean titer before vaccination to the titer after vaccination.

Table 4. Incidence of Local Symptoms.*

Symptom	18–60 Yr of Age		>60 Yr of Age	
	Intramuscular Group (N=69)	Intradermal Group (N=60)	Intramuscular Group (N=50)	Intradermal Group (N=58)
	% of subjects			
Pain†				
Any	67	45	16	19
Grade 2 or 3	32‡	5	6	2
Induration				
Any	6	75§	4	67§
Grade 2 or 3	6	22§	2	3
Redness				
Any	6	88§	8	78§
Grade 2 or 3	5	48§	0	26§
Swelling				
Any	9	52§	4	59§
Grade 2 or 3	1	17§	2	5

* Grade 2 pain was defined as pain when the limb was moved; grade 3 pain was defined as a spontaneously painful limb. Redness, swelling, or induration of grade 2 was defined as ranging from more than 20 mm to no more than 50 mm in diameter, and redness, swelling, or induration of grade 3 was defined as exceeding 50 mm in diameter. Data are presented for 237 subjects for whom data were recorded.

† The severity of pain at the injection site was determined by means of a visual-analogue scale and did not differ significantly between the intramuscular and intradermal subgroups in either the group of subjects 18 to 60 years of age (mean, 10.9 vs. 11.5; $P=0.78$ by Student's *t*-test) or the group of subjects over 60 years of age (mean, 7.1 vs. 7.8; $P=0.70$).

‡ Chi-square=14.3; $P<0.001$ for the comparison with the respective intradermal group.

§ Chi-square=7.0 or greater, depending on the comparison; $P<0.01$ for the comparison with the respective intramuscular group.

geometric mean titer in the intramuscular group to the titer in the intradermal group, 1.8; 95 percent confidence interval, 1.1 to 2.8) (Table 3). Among older subjects, the frequency of seroconversion was less than 30 percent in both the intramuscular and intradermal groups, except in the case of the A/Panama strain in the intramuscular group (39.1 percent) (Table 3); this result may be due to the relatively high antibody levels against each of the strains that were present before vaccination.

No serious adverse events that were related to the vaccines were reported during the study. Local pain, as assessed on diary cards, was significantly less frequent among younger subjects in the intradermal group than among those in the intramuscular group, but among older subjects, between the intradermal and intramuscular groups the frequency did not differ significantly. Local inflammatory responses were significantly more common after

intradermal injection than after intramuscular injection in both age groups (Table 4). These symptoms lasted an average of two or three days and were mainly mild. All resolved without sequelae. In the intradermal group, local reactions consisting of redness, swelling, or induration of more than 50 mm in diameter were the most severe adverse events, occurring in four subjects who were 18 to 60 years of age and in three subjects who were older than 60 years of age. The most severe local reaction reported was 80 mm of redness on day 1 after intradermal vaccination.

DISCUSSION

Intradermal administration of hepatitis B virus or rabies vaccine has been used with moderate success.^{4,5,7-18} The intradermal route has been evaluated in ill persons (such as those with renal failure) in an attempt to improve immunogenicity.^{19,20} In a prospective study of 425 health care workers given 2 µg of hepatitis B surface antigen (HBsAg) by the intradermal route or the standard intramuscular dose of 20 µg, the lower dose given intradermally resulted in antibody titers of more than 10 IU at eight months in 81 percent of subjects, as compared with 93 percent of subjects who were given the full dose intramuscularly.²¹ Slightly larger amounts of HBsAg administered intradermally (one sixth the standard dose) resulted in seroconversion rates that were equivalent to those obtained with a full dose of vaccine administered intramuscularly; however, peak titers were lower (and therefore less durable) than those in the intramuscular group.¹³ The intradermal and intramuscular routes for the prophylactic delivery of rabies vaccine have been compared in schoolchildren in Thailand; three intramuscular injections of 0.5 ml resulted in higher titers than three intradermal injections of 0.1 ml, but there was no significant difference in the percentage of children with titers of at least 0.15 IU per milliliter after the primary series.⁴ Higher titers, however, provided more durable protection at levels above 0.15 IU per milliliter. In general, it appears that lower doses of vaccine given intradermally can provide protective levels of antibody, but that antibody titers of higher magnitude are induced by intramuscular vaccination with larger quantities of antigen, and that these higher titers are more desirable.

The quantity of antigen in the current parenteral influenza vaccine provides moderate protection (efficacy of 40 percent to 90 percent, depending on the

recipient's age²²) against influenza strains that are well matched to vaccine antigens. Relatively high efficacy is observed in healthy young people, who mount vigorous immune responses to 15 µg of antigen given intramuscularly. Older persons often have lower antibody responses and may not be as completely protected as younger persons. Fortunately, among elderly persons, vaccination offers better protection against hospitalization for influenza than against the infection itself, and the use of trivalent inactivated vaccine significantly reduces mortality.²² Strategies to improve the protection afforded by the use of inactivated vaccine include increasing the concentrations of antigens in the vaccine, adding adjuvant to vaccine, and using alternative routes of immunization. In this report, we summarize our experience with the intradermal route.

Although both intramuscular and intradermal vaccination in both age groups met the CPMP criteria, the intradermal route seemed better suited for younger subjects. The level of hemagglutinin antigen in the intradermal dose was reduced to 40 percent of that in the standard intramuscular dose, with equivalent HAI antibody responses in younger persons; less vigorous responses were observed in older persons in both the intradermal and intramuscular groups, and the response to the A/Panama strain (H3N2) in the intradermal group was significantly lower than that in the intramuscular group.

Alternatively, our results may simply reflect the vigorous response of younger persons to smaller quantities of influenza vaccine. Treanor et al. found that half-dose (7.5 µg) influenza vaccine was nearly as immunogenic as full-dose influenza vaccine in healthy young persons.²³ Post hoc analyses of antibody responses in subjects under 65 years of age in the present study revealed no significant differences between the intramuscular group (78 patients) and the intradermal group (78 patients): ratio of the postvaccination geometric mean titer in the intramuscular group to the titer in the intradermal group, 1.1 (95 percent confidence interval, 0.8 to 1.5), $P=0.84$, for the A/New Caledonia strain; 1.2 (95 percent confidence interval, 0.9 to 1.6), $P=0.32$, for the A/Panama strain; 1.0 (95 percent confidence interval, 0.8 to 1.3), $P=0.90$, for the B/Johannesburg strain; and 1.0 (95 percent confidence interval, 0.8 to 1.4), $P=0.80$, for the B/Victoria strain. However, among the subjects who were over 65 years of age, only the responses to the A/Panama strain (H3N2) were significantly lower in the intradermal group

than in the intramuscular group (geometric mean titer ratio, 1.7; 95 percent confidence interval, 1.0 to 3.0; $P=0.03$).

Eighty-eight percent of subjects in the intradermal group who were 18 to 60 years of age had redness at the injection site, 75 percent had induration, and 52 percent had swelling, as compared with less than 10 percent of those in the intramuscular group. Older subjects also had higher rates of local inflammation after intradermal injection than after intramuscular injection. Less than 10 percent of subjects in either age group who underwent intramuscular vaccination had signs of local inflammation. Intradermal vaccination is believed to recruit antigen-presenting cells and macrophages to the skin, and the resulting inflammation may augment the immune response. Future studies should examine cellular responses to intradermal vaccination, since this route may enhance cellular responses. Investigations into the nature of the local inflammatory response should also be considered.

Serum antibody is believed to provide clinically significant protection against influenza. In times of vaccine shortage such as the present, intradermal vaccination of healthy young persons with reduced-dose inactivated influenza vaccine could be considered in order to stretch vaccine supplies. Health care workers and contacts of infants younger than six months old are two groups for which vaccination is recommended, and our results indicate that this approach should induce a sufficient antibody response in the younger members of these groups. Although the intradermal route is more difficult to master and intradermal administration of trivalent inactivated vaccine is not approved for use in the United States, once trained, personnel would be expected to have a high rate of success using standard tuberculin syringes and needles to administer influenza vaccine intradermally at a dose of 6 µg of hemagglutinin per strain.

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REFERENCES

1. Janeway CA Jr, Travers P, Walport M, Capra JD. Immunobiology: the immune system in health and disease. 4th ed. New York: Garland Publishing, 1999.
2. Payler DK, Skirrow MB. Intradermal influenza vaccination. *Br Med J* 1974;2:727.
3. Tauraso NM, Gleckman R, Pedreira FA, Sabbaj J, Yahwak R, Madoff MA. Effect of dosage and route of inoculation upon antigenicity of inactivated influenza virus vaccine (Hong Kong strain) in man. *Bull World Health Organ* 1969;41:507-16.
4. Sabchareon A, Chantavanich P, Pasuraleertsakul S, et al. Persistence of antibodies in children after intradermal or intramuscular administration of preexposure primary and booster immunizations with purified Vero cell rabies vaccine. *Pediatr Infect Dis J* 1998; 17:1001-7.
5. Redfield RR, Innis BR, Scott RM, Cannon HG, Bancroft WH. Clinical evaluation of low-dose intradermally administered hepatitis B virus vaccine: a cost reduction strategy. *JAMA* 1985;254:3203-6.
6. Brown H, Kasel JA, Freeman DM, Moise LD, Grose NP, Couch RB. The immunizing effect of influenza A/NewJersey/76 (Hsw1N1) virus vaccine administered intradermally and intramuscularly to adults. *J Infect Dis* 1977; 136:Suppl:S466-S471.
7. Miller KD, Gibbs RD, Mulligan MM, Nutman TB, Francis DP. Intradermal hepatitis B virus vaccine: immunogenicity and side-effects in adults. *Lancet* 1983;2:1454-6.
8. Ayoola EA, Atoba MA, Johnson AO. Intradermal vaccination against hepatitis B virus infection in an endemic area (Nigeria): two year results. *Arch Virol* 1986;91:291-6.
9. Goldwater PN, Woodfield DG, Ramirez AM, Anzimit IS. Intradermal, low dose, short course hepatitis B vaccination. *N Z Med J* 1986;99:703-5.
10. Milne A, Allwood GK, Pearce NE, Lucas CR, Krugman S. Low dose hepatitis B vaccination in children. *N Z Med J* 1986;99:47-9.
11. Frazer IH, Jones B, Dimitrakakis M, Mackay IR. Intramuscular versus low-dose intradermal hepatitis B vaccine: assessment by humoral and cellular immune response to hepatitis B surface antigen. *Med J Aust* 1987;146:242-5.
12. Wilkins TD, Cossart YE. Low-dose intradermal vaccination of medical and dental students. *Med J Aust* 1990;152:140-3.
13. Henderson EA, Louie TJ, Ramotar K, Ledgerwood D, Hope KM, Kennedy A. Comparison of higher-dose intradermal hepatitis B vaccination to standard intramuscular vaccination of healthcare workers. *Infect Control Hosp Epidemiol* 2000;21:264-9.
14. Jaijaroensup W, Lang J, Thipkong P, et al. Safety and efficacy of purified Vero cell rabies vaccine given intramuscularly and intradermally (results of a prospective randomized trial). *Vaccine* 1998;16:1559-62.
15. Jaijaroensup W, Limusanno S, Khawplod P, et al. Immunogenicity of rabies post-exposure booster injections in subjects who had previously received intradermal pre-exposure vaccination. *J Travel Med* 1999;6: 234-7.
16. Tantawichien T, Banjavongkulchai M, Limsuwan K, et al. Antibody response after a four-site intradermal booster vaccination with cell-culture rabies vaccine. *Clin Infect Dis* 1999;28:1100-3.
17. Briggs DJ, Banzhoff A, Nicolay U, et al. Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. *Bull World Health Organ* 2000;78:693-8.
18. Gherardin AW, Scrimgeour DJ, Lau SC, Phillips MA, Kass RB. Early rabies antibody response to intramuscular booster in previously intradermally immunized travelers using human diploid cell rabies vaccine. *J Travel Med* 2001;8:122-6.
19. Somboonsilp W, Eiam-Ong S, Tungsanga K, Tirawatanapong T. Immune response of intradermal hepatitis B vaccination at lower dose versus intramuscular vaccination at double standard dose in predialytic chronic renal failure patients. *J Med Assoc Thai* 2003; 86:1122-7.
20. Chau KF, Cheng YL, Tsang DN, et al. Efficacy and side effects of intradermal hepatitis B vaccination in CAPD patients: a comparison with the intramuscular vaccination. *Am J Kidney Dis* 2004;43:910-7.
21. Coleman PJ, Shaw FE Jr, Serovich J, Handler SC, Margolis HS. Intradermal hepatitis B vaccination in a large hospital employee population. *Vaccine* 1991;9:723-7.
22. Harper SA, Fukuda K, Uyeki TM, Cox NJ, Bridges CB. Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2004; 53(RR-6):1-40. [Erratum, *MMWR Recomm Rep* 2004;53:743.]
23. Treanor J, Keitel W, Belshe R, et al. Evaluation of a single dose of half strength inactivated influenza vaccine in healthy adults. *Vaccine* 2002;20:1099-105.

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