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Immunogenicity and safety of intradermal versus intramuscular route of influenza immunization in infants less than 6 months of age: A randomized controlled trial

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ABSTRACT

We aimed to explore intradermal influenza vaccination in infants <6 months. One hundred twenty-six infants 2–3 months of age were randomized to receive either two doses, 1 month apart, of 0.25 ml of a trivalent inactivated influenza vaccine (7.5 µg of hemagglutinin per strain) via the intramuscular (IM) route or 0.1 ml of the same vaccine (3 µg of hemagglutinin per strain) via the intradermal (ID) route. The vaccine was well tolerated. Only four infants had hemagglutination inhibition (HAI) titer <40 against ≥1 vaccine-covered antigen pre-vaccination. There was no difference in fold-rise of HAI titer response between those in the IM or ID group. We documented maintenance of HAI titers above seroprotective levels against all three vaccine antigens in 97.6% of subjects regardless of vaccination methods over a time of waning maternal antibodies.

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1. Introduction

Influenza morbidity is high in healthy young children, and highest in children <6 months of age [1–5]. Maternal antibodies were thought to confer full or partial protection to young infants from influenza infection [6]. However, this protection depends on the immunologic experience of the mothers, and with an antigenic drift or shift when the mothers themselves have not experienced the viruses, infants <6 months of age have very severe disease and high mortality [4]. Influenza vaccines are effective in protecting children against influenza but they are believed to be poorly immunogenic in infants <6 months. Seroprotective rates in infants 3–5 months of age after two doses of 0.25 ml (containing 7.5 µg each of the three antigens) of the trivalent influenza vaccine were only 10–55% using four different trivalent vaccines over 4 years in one study [7]. Recently, maternal influenza vaccination at a mean of approximately 2 months before delivery was shown to reduce proven influenza illness by 63% in infants up to 6 months of age [8]. However, during a pandemic, it may not be possible to pre-vaccinate mothers to protect infants. Therefore, it is of utmost importance to explore ways to directly vaccinate infants <6 months for their pro-

tection, both for seasonal influenza as well as during the impending pandemic.

Intradermal (ID) vaccination may be one such means of effectively eliciting good immune responses in infants <6 months while using an acceptable antigen dose. Intradermal vaccination exploits the abundance of antigen-presenting cells (macrophages and dendritic cells) that allow a robust immune response to be elicited with a small dose of antigen delivered directly to the skin, and may be useful to either as a dose-sparing strategy or as a means of improving immunogenicity. Studies of ID administration of influenza vaccine in both adults and children date back to the 1960s [9–13]. Two recent articles reported on the effectiveness of ID influenza vaccination at a reduced dose in adults using the trivalent inactivated influenza vaccine. Both studies documented that ID injection of influenza vaccine was comparable or better than intramuscular (IM) injection of standard dose of the same vaccine [14,15]. We had found that ID vaccination with the trivalent influenza vaccine with one-fifth standard dose was safe and had immunogenicity comparable to the standard dose IM vaccination in 112 healthy children aged 3 to <18 years [16]. A recent Japanese study comparing ID route of administration to subcutaneous administration of two doses of 0.1 ml of influenza vaccine in 6–12 months old infants also demonstrated better immunogenicity [17].

We hypothesized that the ID route of influenza vaccination would result in improved immunogenicity in infants younger than 6 months of age. The current study is the first randomized con-

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trial comparing ID versus IM administration of an inactivated trivalent influenza vaccine in infants 2–3 months of age.

2. Patients and methods

2.1. Study design

This randomized comparison study assessed the safety and immunogenicity of an inactivated influenza vaccine in infants under 6 months of age given via either the IM or ID route in two doses 4 weeks apart. Approval was obtained from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster, and all study activities were conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The parents or legal guardians of all enrolled subjects provided written informed consent prior to any study-related procedures. This study was registered at <http://www.hkclinicaltrials.com> (registration number HKCTR-496).

2.2. Eligibility criteria

Healthy term infants 2–3 months of age at the time of vaccination with uncomplicated birth and no known immunodeficiency or congenital abnormalities were eligible. Exclusion criteria included preterm infants, infants who would be 6 months old at the time of the second dose of vaccine, known or suspected immunodeficiency including exposure to HIV, congenital abnormalities, known allergy to components of the vaccine or receipt of any other vaccine within 5 days.

2.3. Treatment

Subjects who satisfied the entry criteria were matched by age and randomized by a computer generated randomization list (in blocks of 2) to receive either IM administration of the split-dose trivalent inactivated influenza vaccine (Fluarix, GlaxoSmithKline) at 0.25 ml (7.5 μ g of hemagglutinin for each of A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 per dose) or ID administration of the same vaccine at 0.1 ml (3 μ g of hemagglutinin for each of the three antigens per dose). Two doses 4 weeks apart were administered. The dose of 0.25 ml is the same as that recommended for infants 6–35 months of age. The ID dose was limited by the volume feasibly given intradermally to a small infant: 0.1 ml was adopted since this was the volume used in BCG administration and tuberculin skin testing. This dose had been used in our previous study and was also used in a Japanese study in infants <1 year of age [16,17]. The randomization assignment was blinded to the laboratory investigators: blood sent to the laboratory only contained subject identifiers but not treatment information.

2.4. Study procedures

Three to 5 ml of blood were drawn before the first and second doses of vaccination and 21 days after the second dose of vaccination. IM injection was given in the anterolateral thigh and ID injection was given in the deltoid area as previously described [16]. To control for inter-operator variability, the ID vaccination was performed by the same person. The size of induration after ID injection was noted. After each dose of vaccination, subjects were observed for 15–30 min for acute reactions. All vaccination and blood draw were completed by the end of December 2007, well before the start of the winter influenza peak season in Hong Kong to avoid the confounding effect of natural infection.

2.5. Safety assessment

In addition to monitoring immediately after vaccination, caretakers were given a diary card for recording of reactions and adverse effects for 3 days. They were also given a digital thermometer and taught to take temperature by rectal and axilla routes. Solicited reactions were: fever, malaise, shivering, erythema, induration and bruising of the injection site of more than 5 mm in diameter. Side effects were graded according to severity. A mild adverse event was one that the symptoms were easily tolerated, a moderate reaction caused interference with usual activities and a serious one resulted in inability to perform usual activities. Research personnel retrieved the adverse events information on day 4 by telephone. Parents were asked to bring back the diary card for cross-checking when the subjects returned at the following visit.

2.6. Laboratory investigation

Blood samples were collected before the first dose and second dose and 21 days after the second dose of vaccination and were tested for HAI antibody using the 2007–2008 WHO influenza reagent kit from the WHO Collaborating Center for influenza, CDC, Atlanta, Georgia, USA. Specifically HAI antibody assays were performed by standard microtiter techniques after removal of non-specific inhibitors in the serum with receptor destroying enzyme (RDE) (1:3), incubated overnight at 37 °C followed by heat-inactivation at 56 °C for 30 min. All serum samples from each subject were tested in parallel for each of the test antigens. Serial 2-fold dilutions of RDE-treated serum from 1:10 were titrated against four hemagglutinin units of reference antigens using 0.25% turkey erythrocytes in the hemagglutination inhibition assays [16].

For comparison, and to estimate the decline of maternal antibody to an irrelevant antigen, we titrated the first and third blood samples from subjects in parallel for EBV VCA IgG using indirect immunofluorescent technique as previously described [19].

2.7. Statistical analysis

Since the presence of maternal antibody might confuse the seroprotection rate as defined by the percentage of patients with antibody titers ≥ 40 , we elected to use a 4-fold rise in titers to at least one of the influenza antigens as an endpoint. Available data from an early study demonstrated a 4-fold rise in HAI titers against the three antigens in 15–72% of young infants [7,20]. We estimated that approximately 30% of subjects in the IM group would have at least 4-fold rise in HAI titers against any of the antigen contained in the vaccine. Forty-nine subjects in each study group would give 80% power to detect a 30% difference in the percentage of subjects with at least a 4-fold rise in HAI titers against one of the antigens between the two groups, with the percentage of such subjects in the IM group estimated to be 30%. Taking a 20% attrition rate into consideration, we planned to recruit a total of 120 subjects: 60 for each group. Chi-square test was used to compare the adverse events between the ID and IM groups. Paired *t*-test was used to detect any significant difference in vaccine response in geometric mean titres between pre- and post-vaccination blood samples. Unpaired *t*-test was used to compare fold-changes of pre- and post-vaccination blood samples between the ID and IM groups.

To eliminate the confounding effect of natural infection by influenza, parents were instructed to call our research personnel if the subjects develop any signs and symptoms of an acute respiratory infection, i.e., temperature of ≥ 38 °C and cough or runny nose, during the study period. The subject would be seen by the principal investigator and a nasopharyngeal aspirate (NPA) would be obtained for immunofluorescent testing and culture for influenza A and influenza B, and further subtyped if positive.

3. Results

The study started in September 2007 and the last blood draw was completed by the end of December. A total of 126 infants were enrolled. One subject from each group withdrew after the first dose of vaccination and a third subject in the ID group did not return for the final blood draw. The mean ages at the time of the first dose and the third blood draw were (in days): 90.8 ± 18.2 and 143.8 ± 17.6 , and 90.6 ± 18.2 and 143.5 ± 21.7 for the IM and ID group, respectively. Of the 124 intradermal injections performed, the diameter of the weal produced immediately after vaccination was 1–2 mm in 12 and 50.8%, 3–5 mm in 50.85, and >5 mm in 37% of subjects. The influenza vaccine was well tolerated in both groups (Table 1). Mild malaise was reported in a third of subjects regardless of route of administration. Not surprisingly, infants who received the ID vaccination had more redness at the injection site.

Almost all infants had pre-existing HAI antibodies. Only four infants had HAI titer <40 against at least one vaccine-covered antigen before vaccination (Table 2). Of the 123 infants who completed the study, 60 (97.6%) in each group had post-vaccination HAI titers of ≥ 40 to all three antigens in the vaccine (one subject each in the ID and IM group had HAI titer <40 against H1N1, and another subject in the IM group had HAI titer <40 against H3N2). Only eight infants in each group had ≥ 4 -fold rise of HAI titer against one or more antigen (seven infants with one antigen and one infant with two antigens in the IM group and four infants with one antigen, three with two antigens and one with all three antigens in the ID group). There was a significant rise in mean HAI titer against H3N2 after the second dose of vaccine in both groups, and against H1N1 as well in the ID group, but there was a lack of ≥ 4 -fold rise after two doses of vaccination. There was no difference in fold-rise of HAI titer response between those in the IM or ID group (Table 3). While there was no significant fold-rise, the titers against all three antigens remained stable or slightly increased 21 days after the second dose of vaccine in both groups (Fig. 1).

In 2007, there was a summer influenza peak from mid-June to mid-July 2007. Although no infant in this study reported any respi-

ratory illness, to avoid potential confounding by influenza infection of infants born before and during the influenza season, a subgroup analysis was performed on infants born after the influenza season. There were 33 infants (22 in the ID group and 11 in the IM group) with the mean ages at the time of first vaccination dose and third blood draw of 76.8 ± 4.5 days and 131.0 ± 10.4 , and 78.2 ± 7.4 days and 129.4 ± 9.1 days for the IM and ID groups, respectively. They also demonstrated no difference in fold-rise between the IM and ID group but had a significant rise in HAI titer against H3N2 regardless of the route of administration (Table 4). In addition, ID administration resulted in a significant rise of influenza B titer as well in these infants. The scatter plot of the GMT of HAI against each vaccine antigen of these infants is shown (Fig. 2).

In contrast, there was a significant decrease of EBV VCA IgG at 21 days after the second dose of vaccination when compared to that determined in the pre-vaccine serum (Tables 3 and 4, Figs. 1 and 2). EBV was chosen as a surrogate for the fate of maternal antibodies without vaccination or infection because studies showed that 100% of Hong Kong adults are seropositive for this unrelated virus but despite this high prevalence, infection in children does not begin until at least after 8 months of age [18,19]. Here we documented a 40.7% decrease of EBV VCA IgG GMT each month during the 7–8-week study period.

4. Discussion

There are only two published studies in the English literature on influenza vaccination in young infants under 6 months [7,20]. Using the same dose and schedule as the IM group in this study, Groothuis et al. demonstrated seroconversion only in the range of 10–55% against the various vaccine-contained antigens using four different trivalent vaccines over 4 years in 62 infants. A recent phase 1 prospective, open-label study using the same schedule in 42 infants 10–22 weeks of age demonstrated a 4-fold increase of HAI titers in 42.1, 38.9 and 5.3% of 19 subjects for H1N1, H3N2 and B strains included in the vaccine, respectively, in the first season, and in 42.9, 66.7, and 0% of 23 subjects for H1N1, H3N2 and B strains, respec-

Table 1

Adverse events in vaccinated infants during the first 3 days after vaccination of both the first and second dose combined.

Symptoms	Intramuscular, n = 124	Intradermal, n = 124	p-Value
Induration (overall)	3.2%	6.5%	0.374
Mild	3.2%	5.6%	–
Moderate	0%	0.8%	–
Redness (overall)	3.2%	12.1%	0.017
Mild	3.2%	11.3%	0.027
Moderate	0%	0.8%	–
Ecchymosis >0.5 cm	0%	0.8%	–
Malaise (overall)	31.5%	33.1%	0.892
Mild	27.4%	25.0%	0.773
Moderate	3.2%	6.5%	0.375
Severe	0.8%	1.6%	0.561
Shivering (mild)	2.4%	0%	–
Fever >38	2.4%	3.2%	0.701
Irritable	7.3%	11.3%	0.381
Decrease appetite	4.0%	8.1%	0.287

Table 2

Pre- and post-vaccination titres of the four infants with HAI titre <40 for any of the vaccine antigen.

Subject	Route	Pre-vaccination			21 Days post-second dose		
		A/H1N1	A/H3N2	B/Malaysia	A/H1N1	A/H3N2	B/Malaysia
1.	ID	40	20	40	40	20	40
2.	IM	20	20	40	40	40	40
3.	IM	40	20	40	80	40	80
4.	IM	20	40	20	40	20	40

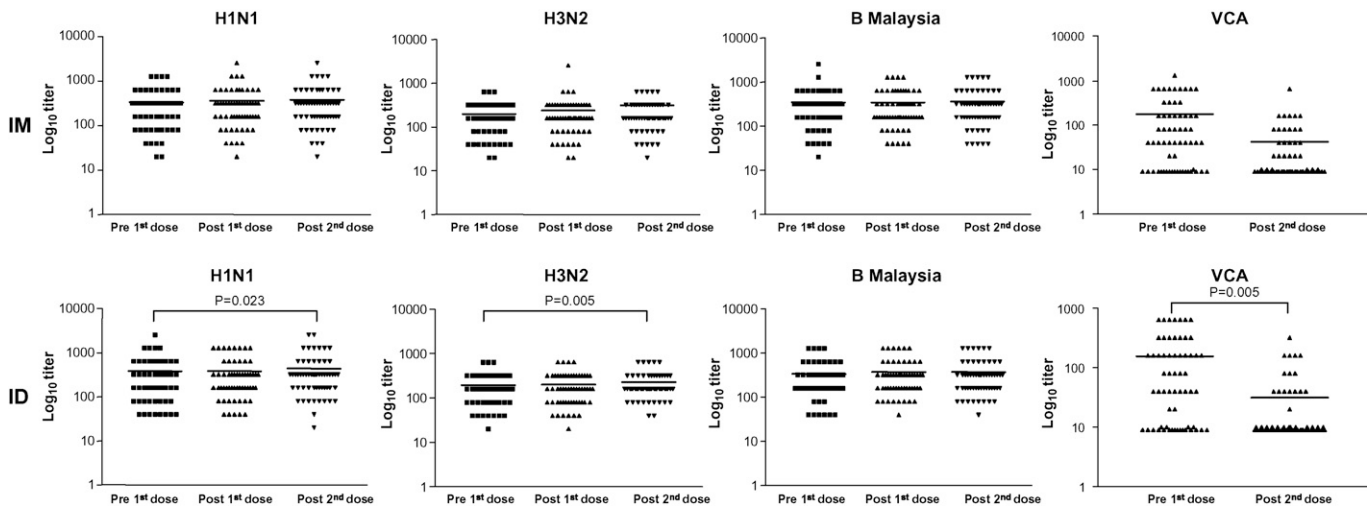


Fig. 1. Geometric mean titers (GMT) of HAI titers all subjects (61 in the IM group and 62 in the ID group) before, after first dose, and 21 days after second dose of influenza vaccination in comparison to the GMT of maternally derived EBV VCA IgG over the same time period.

tively, in the second season [20]. Infants who were seronegative before vaccination were more likely to have a 4-fold rise in antibody titer after vaccination when compared to those who were seropositive. Explanations for such ineffectiveness include effect of maternal antibodies and the immature immune system of young infants.

Intradermal vaccination exploits the abundance of antigen-presenting cells (macrophages and dendritic cells) that allows a robust immune response to be elicited and has been shown to be effective in adults and children [21,22]. This study was planned to examine the possibility of intradermal vaccination as a means of improving immunogenicity in young infants. However, the results surprised us in several ways. It is known that people in Hong Kong are heavily influenza-experienced. We documented one of the highest hospitalization rates for influenza virus infection in children [3]. In another study comparing ID with IM vaccination in children 3 to <18 years, 66, 93.8 and 78.6% of the 112 subjects had protective titers against H1N1, H3N2 and strain B included in the vaccine before vaccination [16]. However, the extremely high percentage of maternal antibodies at protective levels in the infants in this study was still a surprise and somewhat limited our attempt to study influenza vaccine immunogenicity in this age group. The titers were also high,

but were consistent with pre-vaccination titers of older children in our previous study [16]. Our previous study also showed that young children with a high pre-vaccination titer were less likely to respond with at least a 4-fold rise in titer [16]. When compared to the study by Halasa et al using the same dose and regimen as our intramuscular group, they documented a higher percentage of infants with a 4-fold rise of HAI titer against the influenza strains contained in the vaccine. Possible explanations may include more seronegative infants in their study that allowed for a more robust antibody response. Subjects in that study were also older than ours and may have a more mature immune system: mean age of 17.2 weeks compared to 12.9 weeks at enrollment in our study, $p = 0.001$, although it is unclear whether pre-existing maternal antibodies or the infants' immune maturity play a bigger role. However, despite a lack of seroconversion or 4-fold rise, we documented a maintenance and even slight rise of HAI titers over a period of time when maternal antibodies wane. To support this observation, we tested for EBV VCA IgG in parallel and documented a mean decrease of 40.7% per month over the same period of time in these infants. We conclude that the two doses of influenza vaccination were immunogenic and contributed to maintaining the HAI titers to above protective levels. The protective effectiveness against influenza disease by maternal anti-

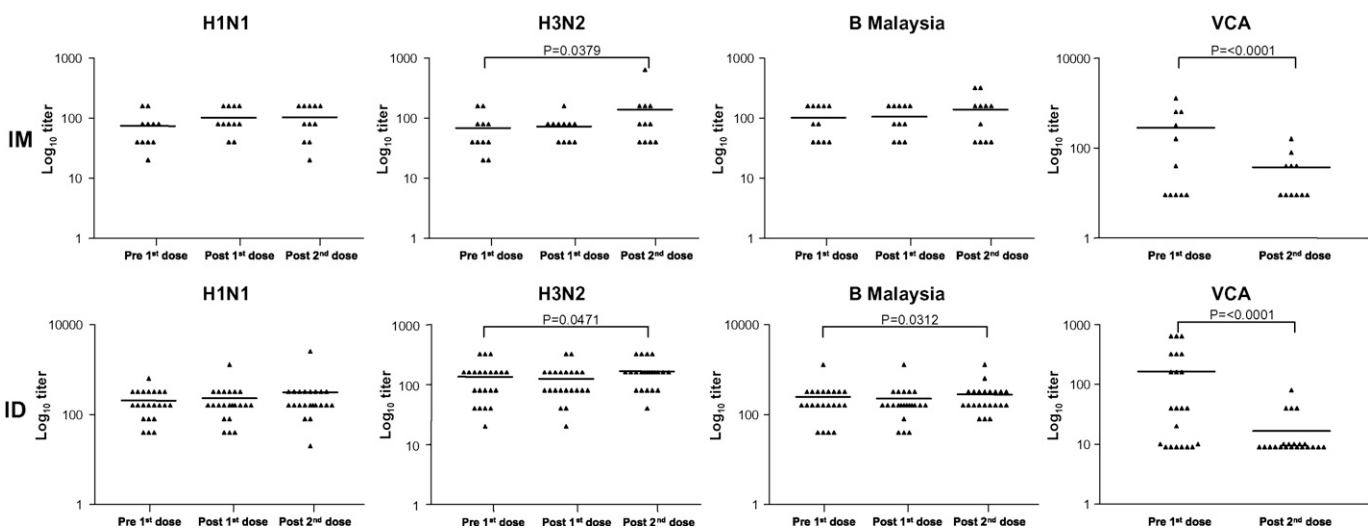


Fig. 2. Geometric mean titers (GMT) of HAI titers subjects born after the influenza peak season (11 in the IM group and 22 in the ID group) before, after first dose, and 21 days after second dose of influenza vaccination in comparison to the GMT of maternally derived EBV VCA IgG over the same time period.

Table 3

HAI titer responses after two doses of influenza vaccination in comparison to maternally derived EBV VCA IgG in all subjects.

		Intradermal route (N = 61)			Intramuscular route (N = 62)			p	
		Pre-vaccination	21 Days post-vaccination	p-Value	Pre-vaccination	21 Days post-vaccination	p-Value		
HAI Geometric Mean Titers (95% CI)	H1N1	229 (173–297)	275 (214–354)	0.023	219 (169–282)	247 (194–316)	0.145	0.552	
	Fold-rise	1.21 (1.02–1.43)			1.13 (0.96–1.34)				
	H3N2	148 (121–180)	185 (157–219)	0.005	148 (119–1183)	186 (147–234)	0.008		0.948
Fold-rise	1.26 (1.07–1.46)			1.26 (1.06–1.50)					
	B Malaysia	236 (188–294)	270 (218–333)	0.122	234 (186–295)	250 (199–314)	0.085	0.477	
	Fold-rise	1.15 (0.99–1.32)			1.07 (0.94–1.22)				
EBV VCA IgG (N = 45)	Fold change	110 (76–161)	15 (12–20)	<0.0001	EBV VCA IgG (N = 43)	127 (87–182)	18 (14–24.3)	<0.0001	0.446
		0.17 (0.13–0.23)			0.20 (0.16–0.24)				

Table 4

HAI titer responses after two doses of influenza vaccination in comparison to maternally derived EBV VCA IgG in infants born after the influenza season.

		Intradermal route (N = 22)			Intramuscular route (N = 11)			p	
		Pre-vaccination	21 Days post-vaccination	p-Value	Pre-vaccination	21 Days post-vaccination	p-Value		
HAI Geometric Mean Titers (95% CI)	H1N1	160 (113–225)	199 (136–292)	0.0897	62 (40–96)	85 (52–138)	0.1762	0.687	
	Fold-rise	1.24 (0.96–1.61)			1.37 (0.85–2.22)				
	H3N2	106 (72–149)	146 (115–185)	0.0471	55 (33–89)	91 (50–162)	0.0379		0.47
Fold-rise	1.37 (1.01–1.87)			1.66 (1.03–2.65)					
	B Malaysia	176 (120–258)	219 (163–295)	0.0312	85 (54–132)	102 (59–180)	0.3409	0.869	
	Fold-rise	1.25 (1.02–1.52)			1.21 (0.79–1.84)				
EBV VCA IgG (N = 16)	Fold change	104 (48–224)	13 (9–17)	<0.0001	EBV VCA IgG (N = 6)	320 (87–1174)	21 (10–44)	<0.0001	0.978
		0.14 (0.07–0.26)			0.14 (0.08–0.23)				

bodies is demonstrated in a recent randomized study that showed a reduction of influenza illness by 63% in infants up to 6 months of age whose mother had received influenza immunization [8].

The summer influenza season in June and July of 2007 introduced the possibility that study subjects who were born during that time could have been infected by influenza and the post-vaccination HAI titers might not be conclusively a result of vaccination alone. Although no parent reported respiratory illness in any of the subjects, we elected to analyze the data both as a whole group as well as only in infants born after the peak influenza season. Findings from this subset correlated with that of the whole study population.

We demonstrated that intramuscular and intradermal vaccination of infants as young as 2 months old using the dosages and regimen in this study is safe and to a certain extent immunogenic. Intradermal vaccination appeared to be more likely to result in higher HAI titers than the intramuscular route: having a significant HAI rise against more antigens included in the vaccine. Previous adult and pediatric studies have shown that one-fifth of the antigenic dose of the inactivated influenza vaccine given intradermally resulted in immunogenicity comparable to that of a full dose given intramuscularly. It is encouraging that the intradermal dose utilized in the current study was only 40% the intramuscular dose (3 µg of hemagglutinin per antigen as compared to 7.5 µg of hemagglutinin per antigen) and yet produced similar, if not better, immunogenic response when compared to the full intramuscular dose. While the fold-rise in HAI titer was only minimal, the lack of waning of titer was of considerable significance, especially when compared to a 40% decrease per month of EBV VCA IgG titer over the same period. Whether using the dose of 7.5 µg each of the three antigens of the vaccine given the intradermal route will result in improved immunogenicity needs to be explored, but this is currently limited by the volume of 0.25 ml. This volume is 2.5 times higher than the standard volume commonly used in intradermal injections. However, a novel microinjection system can deliver 9 or 15 µg of hemagglutinin per strain in 0.1 ml and may offer a possibility for use in young infants [23,24].

In summary, contrary to conventional wisdom, this study demonstrated that in the face of maternal HAI titres, vaccination with the inactivated trivalent influenza vaccine via both the IM and ID routes served to maintain titers above protective levels in infants <6 months of age. Further studies in seronegative infants and with larger doses of intradermal administration should be considered. Specific cellular responses in these infants after influenza vaccination are also under investigation.

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