MAJOR ARTICLE

Live Attenuated and Inactivated Influenza Vaccines in Children

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Background. Live attenuated influenza vaccine (LAIV) and inactivated influenza vaccine (IIV) are available for children. Local and systemic immunity induced by LAIV followed a month later by LAIV and IIV followed by LAIV were investigated with virus recovery after LAIV doses as surrogates for protection against influenza on natural exposure.

Methods. Fifteen children received IIV followed by LAIV, 13 an initial dose of LAIV, and 11 a second dose of LAIV. The studies were done during autumn 2009 and autumn 2010 with the same seasonal vaccine (A/California/07/09 [H1N1], A/Perth/16/09 [H3N2], B/Brisbane/60/08).

Results. Twenty-eight of 39 possible influenza viral strains were recovered after the initial dose of LAIV. When LAIV followed IIV, 21 of 45 viral strains were identified. When compared to primary LAIV infection, the decreased frequency of shedding with the IIV-LAIV schedule was significant ($P = .023$). With LAIV-LAIV, the fewest viral strains were recovered (3/33)—numbers significantly lower ($P < .001$) than shedding after initial LAIV and after IIV-LAIV ($P < .001$). Serum hemagglutination inhibition antibody responses were more frequent after IIV than LAIV ($P = .02$). In contrast, more mucosal immunoglobulin A responses were seen with LAIV.

Conclusions. LAIV priming induces greater inhibition of virus recovery on LAIV challenge than IIV priming. The correlate(s) of protection are the subject of ongoing analysis.

Clinical Trials Registration. NCT01246999.

Keywords. influenza; vaccines; children.

Two distinct approaches exist to prevent influenza in children. Inactivated vaccine (IIV) is injected and recommended for all children aged >6 months. Live attenuated, influenza vaccine (LAIV) is given intranasally and is an approved alternative for healthy children aged >2 years. The evidence for efficacy for IIV in children is more limited than that for LAIV [1]. The development and introduction of LAIV was accompanied by detailed trials that showed a high level of efficacy [2]. Several side-by-side comparisons of the 2 vaccines showed significantly higher efficacy of LAIV than IIV in children in prevention of culture-proven influenza [3–5].

The current study explored permutations of IIV and/or LAIV given as 2 sequential doses 1 month apart. The study was not designed to demonstrate superiority of one vaccine approach over the other but to explore immunity generated by each and to use the frequency and extent of virus shedding with a 1-month LAIV challenge as a benchmark of short-term immune protection.

METHODS

The study was done collaboratively between the Vaccine Research Unit at the University of Rochester Medical

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Center and the Geisel School of Medicine at Dartmouth with the support of the Laboratory of Infectious Diseases of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health. Children were recruited from the Rochester community and the Children's Hospital at Dartmouth pediatric clinics in Lebanon and Manchester, New Hampshire. The first year of the study was conducted between 5 October 2010 and 31 January 2011. The second year of the study was conducted between 27 September 2011 and 1 February 2012. Although final sera were collected during the winter influenza season, there was limited and seasonally late influenza circulation in both years. The children were under surveillance and no influenza-like illness was reported in participants in either year.

Informed assent/consent was obtained from all participants and their parents using protocols and consent forms approved by each institutional investigational review board. The conduct of the study was monitored by the Regulatory Compliance and Human Subjects Protection Branch of NIAID.

Study Design

Children in the first year were randomly assigned to receive (1) LAIV intranasally twice, (2) IIV intramuscularly twice, (3) LAIV intranasally followed by IIV intramuscularly, or (4) IIV intramuscularly followed by LAIV intranasally. In the second year, randomization was confined to the 2 groups of greatest interest: (1) LAIV intranasally twice and (2) IIV intramuscularly followed by LAIV intranasally. No child received other influenza vaccination during enrollment in the study. Children with specified underlying illnesses were excluded. Safety of vaccination was assessed using symptoms collected by parents for 7 days after each dose of the vaccine.

Blood samples were obtained prior to and 28 days following each vaccine dose and assessed for the presence of serum antibodies. Cellular immune responses were measured; however, none were convincingly positive (for methods and results, see the Supplementary Data).

Samples for measurement of secretory antibodies were obtained by insertion of nasal wicks for 2 minutes prior to and on day 28 after each vaccine dose for immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies measured by kinetic enzyme-linked immunosorbent assay (kELISA) and Luminex assay.

Nasal swab specimens obtained on days 0, 2, 4, and 7 after each dose of LAIV were assessed for the presence and magnitude of shedding of each strain of vaccine virus.

Vaccines and Viruses

Each vaccine contained the influenza strains matching the antigens recommended by the US Food and Drug Administration, which for both the 2010–2011 and 2011–2012 influenza seasons were A/California/07/09 (H1N1), A/Perth/16/09 (H3N2), and B/Brisbane/60/08.

Immunologic Assays

Serology

Serum antibody responses to vaccines were determined using hemagglutination inhibition (HAI), microneutralization (MN), and neuraminidase inhibition (NAI) assays as described previously [6]. Subjects were considered seronegative if their serum before vaccination had an HAI titer $\leq$1:4. The viruses used for the NAI had the vaccine strain neuraminidase, an irrelevant hemagglutination (A/teal/HK/97 [H6] in the case of influenza A and B/Ann Arbor/1/66 in the case of influenza B) with PR8 or B/Ann Arbor/1/66 internal genes (kindly supplied by Hong Jin, Medimmune, Gaithersburg, Maryland). Seroresponses to vaccine antigens were defined as $\geq$4-fold rises in antibody titer between appropriate paired specimens. Serologic responses to the first dose were calculated as changes between days 0 and 28 and for the second dose those between days 28 and 56.

Virus-neutralizing titers were determined by infection of Madin–Darby canine kidney (MDCK) cells and expressed as the reciprocal of the highest serum dilution that neutralized 50% of 100 50% tissue culture infectious doses ($TCID_{50}$) of virus after incubation at 37°C for 72 hours.

Kinetic ELISAs

Sera were tested for IgA and IgG antibodies to the inactivated vaccine viruses A/California/07/09 (H1N1), A/Perth/16/09 (H3N2), and B/Brisbane/60/08 (kindly donated by Novartis, Liverpool, United Kingdom). The vaccine preparations are based on a standard amount of HA and variable quantities of NA. Immulon 2 plates were coated with vaccine virus corresponding to the antigens contained in the vaccines and kELISA was performed, as previously described [7]. Nasal wick samples were also tested using the same antigens to measure vaccine-specific IgA and IgG, which were expressed as a fraction of the total IgA or IgG in the nasal wick specimen. The kELISA responses correlated well with those to a Luminex-based assay (Supplementary Data).

Virologic Assays

Viral Isolation and Identification

Nasal swab specimens were tested for vaccine viruses by quantitative viral culture in MDCK cells at 33°C and by quantitative reverse transcription polymerase chain reaction (qRT-PCR) amplification [8]. The limit of viral detection was $10^{0.6}$ $TCID_{50}$/mL for virus culture and $10^{0.4}$ $TCID_{50}$/mL for qRT-PCR.

Influenza Strain Identification by Plaque Assay

To identify each strain present in nasal swab specimens, plaque assays were performed in the presence of human antisera specific for the components of influenza vaccine [9]. In brief, confluent monolayers of MDCK cells were infected with nasal specimens collected by a nasopharyngeal swab placed in 1 mL of viral transport media. After absorption for 1 hour at 33°C, cells were

overlaid with minimal essential medium containing 0.3% bovine serum albumin, 0.9% Bacto agar, and 1 mg/mL L-[tosyl amido-2-phenyl] ethyl chloromethyl ketone–treated trypsin. After 3 days of incubation at 33°C, cells were fixed with 4% paraformaldehyde and subtype-specific human antisera (kindly donated by MedImmune), followed by staining with peroxidase-conjugated rabbit antisheep antibodies (KPL, Gaithersburg, Maryland). Plaques were developed with 3,3′-diaminobenzidine substrate (Vector Laboratories, Burlingame, California) prepared according to the manufacturer's instructions. Plaque assays were done in triplicate.

Statistical Analysis

The analysis is based primarily on 11 children who received LAIV followed by LAIV and the 15 children who received IIV followed by LAIV. Differences in the response to vaccination with either a single dose of LAIV, 2 doses of LAIV, or IIV followed by LAIV were evaluated using Poisson regression to estimate the effect of vaccination regimen on viral shedding. The dependent variable was the number of viruses recovered in culture at each time point and for each viral subtype. Linear and quadratic terms were included to model and adjust for the effect of time on viral shedding patterns, and an offset term was included to account for the total possible number of recovered viruses. For key analyses of shedding and immune response, the data are shown for individual strains as well as in aggregate. Cluster-robust standard errors were calculated to adjust for any nonindependence of shedding counts within a given viral subtype. A Bonferroni correction was made to address multiple testing in postestimation hypothesis testing. Associations between antibody responses and viral shedding and between antibody responses and age were evaluated using Pearson correlation test. Vaccine regimen–specific differences in proportions of viral isolates recovered were evaluated using Fisher exact test.

RESULTS

Study Enrollment

Thirty-four participants were enrolled (17 boys and 17 girls), ranging in age from 2 years and 3 months to 9 years and 8 months. Three were black, 30 were white, and 1 was biracial.

The mean age of children initially receiving LAIV was 69 months and the mean age of children receiving IIV was 64 months. Data from 5 additional children who received IIV followed by IIV (n = 3) or LAIV followed by IIV (n = 2) are not presented except for responses to their first dose that contribute to the analysis. Three children did not complete the study. Of these, 2 were hesitant to continue because of the procedures involved and 1 was lost to follow-up.

Vaccine Safety

Both vaccines were given in age groups and under conditions approved for their routine seasonal use. The vaccines were well tolerated with only minor reactions (Supplementary Data).

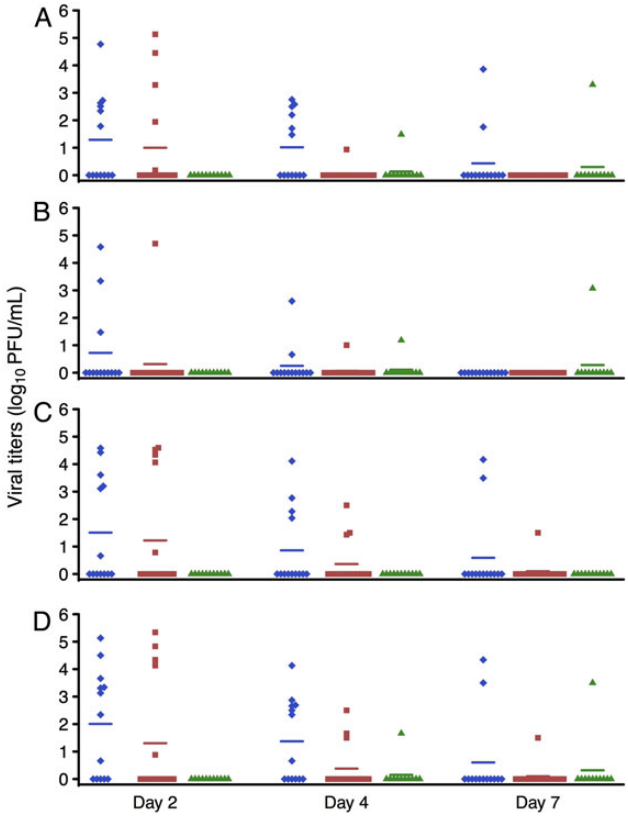

Figure 1. Amount of virus recovered by strain with different schedules. *A–C*, Titer of virus recovered on days 2, 4, and 7 with influenza H1N1, H3N2, and *B*, respectively, after trivalent live attenuated influenza virus (LAIV). The response to initial dose of LAIV is shown in the blue diamonds. The response to LAIV given after inactivated influenza vaccine is represented by red squares and the response to a second dose of LAIV by green triangles. Horizontal lines represent the geometric mean titer of virus shed, and the continuous horizontal dotted line represents the lower limit of detection with negative values assigned a value one-half the lower limit of detection. *D*, Composite of all strains recovered. When values were identical, they are shown as superimposed on each other. Abbreviation: PFU, plaque-forming unit.

LAIV Shedding (First Dose)

Of 13 children receiving LAIV as the first dose of vaccine, 9 shed H1N1, 9 shed H3N2, and 10 shed influenza B. Thus, replication of 28 among 39 possible strains (72%) was detected. Three of 13 had no influenza recovered after vaccination. The peak titer of virus recovered by strain is shown in Figure 1*A–C*. The aggregate virus shedding is shown in Figure 1*D*. The most children were shedding virus on day 2 for all 3 strains (Figure 2).

Virologic responses were not influenced by age and there was no influence of preexisting HAI antibody titer on the likelihood of LAIV virus shedding after the first dose of LAIV (Table 1). This was true in comparisons between seronegative (≤1:4) and seropositive (>4) subjects and in comparisons with seronegative subjects and those with titers ≥1:32. When preexisting HAI

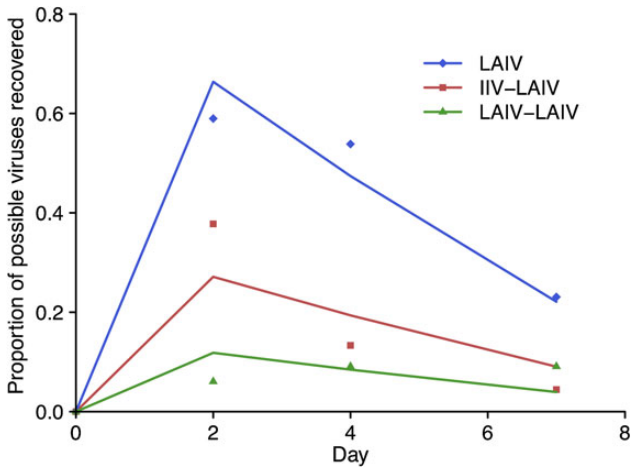

Figure 2. Viral shedding over time by vaccination regimen. Points indicate the percentage of viruses of any subtype recovered in culture at each time point for each vaccination group. Lines indicate Poisson regression estimates, which demonstrate that live attenuated influenza virus (LAIV) shedding with the initial dose (blue line) is significantly greater than after the second LAIV dose (green line) ($P < .001$); that LAIV shedding after inactivated influenza vaccine (IIV; red line) is significantly lower than after the initial LAIV dose (blue line) ($P < .001$), and that LAIV shedding after LAIV (green line) and after IIV (red line) are not significantly different ($P = .098$).

antibody was plotted by age, there was an increase in HAI titer with age only to influenza B virus (Spearman correlation coefficient = 0.46, $P = .007$; Figure 3). In further analyses, the responses to vaccination were viewed without regard to evidence of prior infection. Correlations between the immune parameters measured and protection afforded on challenge will be the subject of a separate publication.

Immune Responses (First Dose)

If preexisting immunity is considered to be an HAI titer of ≥1:4, only 9 of 31 subjects were seronegative to H1N1 (4/13 LAIV recipients and 5/18 IIV recipients), 8 of 31 were seronegative to H3N2 (2/13 LAIV recipients and 6/18 IIV recipients), and 8 of 31 were seronegative to B (3/13 in the LAIV recipients and 5/18 in the IIV recipients). The nature of the prior influenza exposure (natural infection, LAIV, and/or IIV) could not be determined.

The responses to IIV and LAIV differed. IIV induced significantly more humoral immune responses after the first dose of vaccine than LAIV (Table 2). The ability to mount an HAI response was not influenced by age or preexisting antibody (data not shown). Following a single dose of LAIV, antineuraminidase responses were rarely seen (Table 2). Mucosal IgG responses were seen more consistently with IIV than LAIV (Table 2) and correlated with rises in serum IgG (data not shown). By kELISA, the frequency of mucosal IgA responses to LAIV (14/39 [36%]) was marginally greater than that to IIV (10/54 [19%]; $P = .09$). Luminex IgA responses are shown in the Supplementary Data and corroborate the kELISA data.

Effect of IIV on 1-Month LAIV Challenge

In 15 children challenged with LAIV 1 month after receipt of IIV, H1N1 virus was recovered from 6 children, H3N2 from 5, and B from 10. Thus, 21 of 45 (47%) possible strains were recovered despite prior IIV. No virus was recovered from 4 of 15 children. When compared with the response to the initial dose of LAIV, IIV provided marginal inhibition of subsequent shedding of H1N1 ($P = .07$) and H3N2 ($P = .07$), but none against influenza B ($P = .95$) (Table 1). By Poisson regression analysis, the shedding of influenza strains was significantly reduced when compared to the initial LAIV dose (Figure 2). The titers on days 2, 4, and 7 in those who shed virus are shown in Figure 1*A–D*. This temporal analysis suggests that LAIV shedding after IIV may be truncated after day 2.

IIV-LAIV induced virtually no additional systemic responses, but led to mucosal IgA responses in 24% of children and mucosal IgG responses in 20% of children (Table 2).

Effect of LAIV on Subsequent Challenge With LAIV

The shedding of LAIV was reduced in those who had previously received LAIV with identifiable H1N1, H3N2, and B viruses recovered from only 1 child (green triangles, Figures 1*A–D* and 2). The 3 viruses identified were all from a single, healthy 4-year-old child who had also shed all 3 strains with the initial dose of LAIV but had very limited mucosal or serum responses to any of the 3 vaccine strains with the initial dose. However, with the second dose, the child mounted a systemic immune response to H3N2 by HAI. The second dose of LAIV induced minimal additional systemic or mucosal responses.

Alternative Immunization Strategies

In year 1, a dose of IIV was given to 3 children who had previously received IIV and 2 children who had previously received LAIV. In both settings there were few boosts in mucosal or systemic antibody titers with the second dose. These 2 arms of the study were discontinued in the second year.

DISCUSSION

The study was designed to define differences in the nature and extent of immune responses to IIV and LAIV and to determine protection afforded by each vaccine, using LAIV as a surrogate for wild-type influenza virus. The assumption is made that protection against virus shedding on short-term LAIV challenge may be relevant to a component of immunity that relates to the ability of vaccination to create a herd effect and prevent community-wide spread of disease.

Four studies have compared the effectiveness of IIV vs LAIV in a direct challenge model. The first was carried out in adults

Table 1. Live Attenuated Influenza Virus Shedding

Vaccine		Total No. of Subjects	Influenza Vaccine Strain	Prevccination HAI[a]	No. of Subjects With Respective HAI	Virus Recovery by Culture or PCR (No. of Subjects by Strain)	Geometric Mean Titers	
First Dose	Second Dose						Prevaccination	Postvaccination
LAIV	. . .	13	A/California/07/09 (H1N1)	>1:4	9	6 (67%)	75	75
				≥1:32	6	3 (50%)	203	203
				≤1:4	4	3 (75%)	3	3
			A/Perth/16/09 (H3N2)	>1:4	11	8 (72%)	39	120
				≥1:32	6	4 (67%)	128	161
				≤1:4	2	1 (50%)	3	45
			B/Brisbane/60/08	>1:4	10	7 (70%)	17	37
				≥1:32	3	1 (33%)	40	80
				≤1:4	3	3 (100%)	3	25
			Total (out of 39)	>1:4	30	21 (70%)	36	70
				≥1:32	15	8 (53%)	122	154
				≤1:4	9	7 (78%)	3	12
LAIV	*LAIV*	11	A/California/07/09 (H1N1)	>1:4	8	1 (13%)	83	76
				≥1:32	5	0 (0%)	333	382
				≤1:4	3	0 (0%)	3	4
			A/Perth/16/09 (H3N2)	>1:4	11	1 (9%)	100	128
				≥1:32	8	0 (0%)	215	197
				≤1:4	0	NA	NA	NA
			B/Brisbane/60/08	>1:4	11	1 (9%)	39	39
				≥1:32	7	0 (0%)	86	78
				≤1:4	0	NA	NA	NA
			Total (out of 33)[b]	>1:4	30	3 (10%)	67	72
				≥1:32	20	0 (0%)	169	152
				≤1:4	3	0 (0%)	3	4
IIV	*LAIV*	15	A/California/07/09 (H1N1)	>1:4	15	6 (40%)	645	489
				≥1:32	15	6 (40%)	645	489
				≤1:4	0	NA	NA	NA
			A/Perth/16/09 (H3N2)	>1:4	13	5 (39%)	207	150
				≥1:32	11	4 (36%)	329	226
				≤1:4	2	0 (0%)	3	64
			B/Brisbane/60/08	>1:4	15	10 (67%)	111	102
				≥1:32	11	8 (73%)	256	226
				≤1:4	0	NA	NA	NA

Table 1 continued.

Vaccine		Total No. of Subjects	Influenza Vaccine Strain	Prevccination HAI[a]	No. of Subjects With Respective HAI	Virus Recovery by Culture or PCR (No. of Subjects by Strain)	Geometric Mean Titers	
First Dose	Second Dose						Prevaccination	Postvaccination
		Total (out of 45)		>1:4	43	21 (49%)	248	198
				≥1:32	37	18 (49%)	401	309
				≤1:4	2	0 (0%)	3	64

Shedding occurred after the vaccine dose shown in italics.

Abbreviations: HAI, hemagglutination inhibition; IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; NA, not available; PCR, polymerase chain reaction.

[a] Subjects with prevaccination HAI titers >1:4 include patients with prevaccination HAI titers ≥1:32.

[b] Shedding of identifiable H1N1, H3N2, and B viruses were all from the same child. This child had a mucosal kinetic enzyme-linked immunosorbent assay immunoglobulin A response to influenza A(H1) and B with the first dose as well as a neuraminidase inhibition response to influenza B. With the second dose, the child had an HAI and microneutralization response to influenza A(H3N2).

Figure 3. The magnitude of hemagglutination inhibition (HAI) titer as a function of age at time of vaccination is shown with only influenza B showing a significant rise with age. H1 vs age: Pearson correlation coefficient = 0.11; $P = .540$; H3 vs age: Pearson correlation coefficient = 0.31; $P = .080$; B vs age: Pearson correlation coefficient = 0.46; $P = .007$.

with experimentally administered challenge with wild-type virus. This study, like the current study, identified greater protection against shedding from LAIV than IIV [10] and defined different correlates of protection for the 2 vaccines [11]. The second study employed a wild-type challenge of adults with a strain to which they were initially susceptible (HAI ≤1:8) 1 month after placebo, LAIV, or IIV [12]. In this study no significant differences in virus recovery were seen between any of the 3 groups on challenge, and estimates of vaccine efficacy were not different between LAIV and IIV. In the third study, children vaccinated with LAIV or IIV were challenged 1 year later with LAIV. Similar to the current study, LAIV provided greater protection against virus shedding than IIV even 1 year after vaccination [13]. The fourth study had a similar design and age group to the present study and examined virus shedding and humoral and T-cell responses to sequences of LAIV and IIV influenza vaccines [14]. Although virus shedding was not quantitated and was documented only by culture, the results are similar to the present study with 17 of 28 subjects shedding virus after the first dose of LAIV, and 1 of 13 after the sequence of LAIV-LAIV, and 4 of 13 after IIV-LAIV. The study by Hoft et al [14] suggested that cellular immunity may be a key difference in the immune response between LAIV and IIV. We could not replicate those cytotoxic T lymphocyte findings in the current study (see Supplementary Data). Additionally, none of these studies looked with the detail of the present study at the immune profile generated by the 2 vaccines.

Placebo-controlled studies at the time of introduction of LAIV showed efficacy >90% either with natural infection (H3N2 and B) [2] or vaccine challenge (H1N1) [15]. Although widely recommended for children and with an efficacy estimated at 40%–60% from observational studies, definitive studies of efficacy of IIV are lacking [16, 17].

Table 2. Immune Responses to Vaccine Dose

Vaccine				No. of Subjects With ≥4-Fold Increase in Titer			
First Dose	Second Dose	Total No. of Subjects	Parameters of Immunity	A/California/07/09 (H1N1)	A/Perth/16/09 (H3N2)	B/Brisbane/ 60/08	No. (%) of Responses (All Strains)
LAIV	. . .	13	HAI	0	5	6	11/39 (28)[a]
			NAI	0	1	2	3/39 (8)[b]
			MN	0	5	5	10/39 (26)[c]
			IgG Sys	1	5	4	11/39 (33)[d]
			IgA Sys	0	1	3	9/39 (23)
			IgG Muc	2	3	5	10/39 (26)[e]
			IgA Muc	3	5	6	14/39 (36)[f]
IIV	. . .	18	HAI	16	11	13	40/54 (74)[a]
			NAI	7	1	6	14/54 (26)[b]
			MN	12	13	9	34/54 (63)[c]
			IgG Sys	5	13	13	31/54 (57)[d]
			IgA Sys	2	2	6	10/54 (19)
			IgG Muc	9	12	8	29/54 (54)[e]
			IgA Muc	7	1	2	10/54 (19)[f]
LAIV	*LAIV*	11	HAI	0	1	0	1/33 (3)
			NAI	0	0	1	1/33 (3)
			MN	0	2	0	2/33 (6)
			IgG Sys	1	0	0	1/33 (3)
			IgA Sys	0	0	0	0/33 (0)
			IgG Muc	2	0	3	5/33 (15)
			IgA Muc	1	0	0	1/33 (3)[g]
IIV	*LAIV*	15	HAI	1	1	0	2/45 (4)
			NAI	1	1	3	5/45 (11)
			MN	0	1	1	2/45 (4)
			IgG Sys	1	1	2	2/45 (4)
			IgA Sys	0	1	0	1/45 (2)
			IgG Muc	3	2	4	9/45 (20)
			IgA Muc	3	1	7	11/45 (24)[g]

Immune response occurred after dose shown in italics.

Abbreviations: HAI, hemagglutination inhibition; IgA, immunoglobulin A; IgG, immunoglobulin G; IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; MN, microneutralization; Muc, mucosal response by kinetic enzyme-linked immunosorbent assay; NA, not available; NAI, neuraminidase inhibition; PCR, polymerase chain reaction; Sys, systemic response by kinetic enzyme-linked immunosorbent assay.

[a] $P < .001$.

[b] $P = .03$.

[c] $P < .001$.

[d] $P = .006$.

[e] $P = .01$.

[f] $P = .09$.

[g] $P = .01$.

LAIV stood out as providing greater inhibition of virus replication under the conditions of our study. Placebo-controlled studies at the time of introduction of LAIV showed efficacy >90% either as a result of natural infection (H3N2 and B) [2] or vaccine challenge (H1N1) [15]. Differences were readily demonstrated in the immune response to IIV as opposed to LAIV, with the former the more immunogenic by all humoral criteria measured. Induction of mucosal antibodies was inconsistent but more prominent to LAIV than IIV as measured by kELISA and by Luminex.

The age of the volunteers was such that many had exposure to influenza through IIV or LAIV vaccination or wild-type infection. When volunteers were divided by HAI serostatus, there was no statistical relationship of an HAI titer of ≤1:4 (7/9 shedding), >1:4 (21/30 shedding) or even ≥1:32 (8/15 shedding) with virus recovery following an initial dose of LAIV. There

are limitations of sample size, but one interpretation might be that the key correlate(s) of immunity to influenza do not lie in the parameters of immunity usually measured to reflect protection—this despite the fact that murine studies have shown quite clearly that IgA antibody protects the mucosal surface of the upper respiratory tract and IgG has a similar role in the lung [18].

A number of the immune responses were significantly different between IIV and LAIV (Table 2). Serum responses with HAI, MN, and serum IgG were more striking with a single dose of IIV. Mucosal IgA responses as measured by kELISA were greater with LAIV than IIV. There was evidence that the administration of IIV followed by LAIV enhanced the mucosal antibody response after LAIV shedding in some of prior recipients of IIV. The serum antibody response in this age group to a single dose of IIV was high and there were no further immune responses with the second dose in 3 children who received 2 doses of IIV. Cellular immunity was not detected with either vaccine under the conditions of the study. The frequency and volume of blood that could be drawn did not permit examination of plasmablasts and activated T cells at the peak of the cellular immune response, which typically occurs on days 8–10 [19].

The shedding with a second dose of LAIV was strikingly diminished when compared with that seen in the children whose initial vaccine exposure had been IIV. The implications of diminished but still substantial LAIV shedding after IIV are not immediately apparent. Does this mean that IIV will provide less community-wide herd protection? Is it an effect that persists for 6 months or more, with resultant implications for the influenza season, or is it a short-term effect just measurable at the 1-month interval at which challenge was done?

There is evidence both for and against the reduced shedding observed being due to short-term nonspecific or innate immunity. Older studies have shown heterotypic immunity between different rhinovirus strains that was present at 5 weeks but not at 16 weeks [20], suggesting that heterotypic immunity could be seen with viruses. However, arguing against a short-term nonspecific immunity is the study of Johnson et al, which showed a similar pattern to the present study with prior LAIV being highly protective and IIV not protective when the LAIV challenge was 1 year after the initial vaccination [13].

The assumption going into the trial was that mucosal antibody would be the key determinant of immunity, as has been shown for parainfluenza type 1 [21]. It is clear from the data analyzed to date that correlates of immunity will be difficult to derive, but such an effort is ongoing. Also noted was that the 1 child who shed each virus strain with the second LAIV dose had shed all 3 strains with the first dose. Particular attention was paid to this child. Although the responses seemed blunted, there were no obvious deficits in this child's immune response to influenza.

There were some limitations to this study. The study was not blinded or placebo controlled. This was done in an effort to assure that volunteers received appropriate protection against influenza for the upcoming season. Although recommendations in this age group call for 2 doses of vaccine to achieve full immunity if vaccine has not previously been given, the high level of preexisting immunity and strong systemic immune response to the first IIV dose suggest that the relatively poor protection afforded by a single dose of IIV on LAIV challenge would not have been improved after 2 doses.

The study was done over 2 years to enroll a sufficient number of volunteers. The components of the vaccine remained unchanged, and the intervening winter of 2010–2011 was not severe in terms of influenza penetrance.

Some strain-specific differences were seen: a lack of systemic responses to H1N1 in LAIV, an absent NAI response to H3N2 in IIV, and no protection from influenza B in IIV on subsequent challenge with LAIV. With the small population enrolled and multiple immunologic assays performed, it is not possible to generalize from these observations.

In conclusion, this study demonstrates that LAIV provides greater short-term inhibition of vaccine virus shedding than IIV even though IIV is the more immunogenic product by most parameters measured. The duration of enhanced protection by LAIV can only be speculated, but it seems unlikely that the protection afforded by IIV would improve over time.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No potential conflicts of interest.

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