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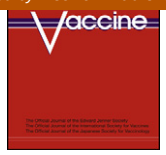

The role of nasal IgA in children vaccinated with live attenuated influenza vaccine

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ABSTRACT

Background: Immunoglobulin A (IgA) is the predominant antibody produced in response to mucosal infections. The role of IgA in providing protection against influenza in children vaccinated with live attenuated influenza vaccine (LAIV) has not been well described.
Methods: Nasal IgA responses were assessed using data from 3 prospective, 2-year, randomized studies comparing LAIV with placebo in children 6–36 months of age. In each study, samples were collected in a subset of patients; a new cohort was enrolled each year. Ratios of strain-specific nasal IgA to total nasal IgA were calculated and prevaccination to postvaccination geometric mean fold-rises (GMFRs) were evaluated. Mean postvaccination IgA ratios were compared for subjects with and without confirmed influenza illness by study and in pooled analyses.
Results: Across studies, a higher percentage of children receiving LAIV had a ≥2-fold increase in strain-specific IgA ratio compared with placebo recipients. GMFRs after LAIV in years 1 and 2 ranged from 1.2 to 6.2, compared with 0.5–2.2 among placebo recipients. Similar responses were observed in subjects who were baseline seronegative and seropositive based on serum hemagglutination inhibition antibody titers. In years 1 and 2, the mean postvaccination strain-specific to total IgA ratio was 3.1-fold ($P < 0.01$) and 2.0-fold ($P < 0.03$) higher among LAIV recipients with no evidence of culture-confirmed influenza illness compared with LAIV recipients who developed culture-confirmed influenza illness; a similar and consistent trend was observed for each individual study and type/subtype.
Conclusions: The current analysis demonstrates that nasal IgA contributes to the efficacy of LAIV and can provide evidence of vaccine-induced immunity. However, the inherent heterogeneity in nasal antibody levels and variability in nasal specimen collection hinders the precise evaluation of mucosal antibody responses. Other studies have demonstrated that LAIV-induced immunity is also partially explained by T-cell immunity, serum antibody responses, and innate immunity, consistent with the multi-faceted nature of immunity induced by wild-type influenza infection and other live virus vaccines.
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1. Introduction

Infection with wild-type influenza induces immunity to subsequent infection with antigenically related strains primarily through serum and mucosal antibodies. While serum antibodies are generally responsible for lower respiratory tract protection, local mucosal antibodies are critical for protection of the upper respiratory tract. T-cell and innate immune responses also contribute to protection and reductions in illness severity [1–3]. In order to prevent influenza illness, vaccination has long been established as the preferred approach [4].

An Ann Arbor strain live attenuated influenza vaccine (LAIV; MedImmune, LLC, Gaithersburg, MD) is licensed for use in a number of countries in eligible individuals 2–49 years of age [5]; in the European Union, LAIV is approved for use in children 2–17 years of age; in Canada, LAIV is approved for individuals 2–59 years of age. LAIV has been shown to be effective in preventing culture-confirmed influenza illness in children and adults [6–8]; in children, studies have demonstrated that LAIV provides greater protection than standard inactivated influenza vaccines [9–12]. However, despite multiple immunologic investigations, robust immunologic correlates of protection have not been established for LAIV.

Although functional serum antibody titers as measured by hemagglutination inhibition (HAI) are generally regarded as the correlate of protection for inactivated influenza vaccines, the general trend observed in studies of LAIV-induced immune responses is that adults demonstrate limited serum antibody responses to LAIV; by comparison, young children, particularly those without

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pre-existing antibodies, can exhibit higher rates of seroconversion in response to vaccination [13–21]. Studies have demonstrated that LAIV can induce protective immunity in the absence of robust serum antibody responses [22–25]. Studies have also demonstrated that LAIV induces mucosal antibody responses [26,27] and T-cell responses [17,28–30] that may contribute to protective immunity.

Immunoglobulin A (IgA) is the predominant antibody at mucosal surfaces, with both extracellular and intracellular activity [31], and high levels of anti-influenza antibody secreting cells have been demonstrated in the nasal mucosa in adults with a history of previous wild-type influenza exposures [32]. A recent study of children with severe influenza disease suggested that anti-influenza mucosal antibody may be particularly important in children [33]. There is also evidence that IgA may be more cross-reactive against antigenically drifted influenza viruses than IgG [34].

Although a previous study demonstrated IgA responses following LAIV, the relationship between IgA responses and the incidence of influenza illness was not evaluated [27]. Three previous randomized, placebo-controlled clinical studies of LAIV efficacy in young children prospectively evaluated postvaccination IgA responses in a subset of study subjects [14,20,35]. This analysis describes the strain-specific IgA responses observed in these 3 studies and examines the relationship between IgA and the incidence of influenza illness.

2. Methods

2.1. Subjects

Nasal IgA responses were evaluated using data from 3 prospective, 2-year, randomized, placebo-controlled studies of LAIV in children. The detailed methods and inclusion/exclusion criteria for each study have been previously published. Study 1 was a 2-year study conducted in influenza vaccine-naive children 12 to <36 months of age from 2000 to 2002 in Asia [20]. Study 2 [35] was conducted in influenza vaccine-naive children 6 to <36 months of age attending day care in several European countries and Israel from 2000 to 2002. Study 3 [14] was conducted in influenza vaccine-naive children 6 to <36 months of age in South America and South Africa in 2001–2002. In studies 1 and 2, children were randomized to 2 doses of vaccine or placebo approximately 1 month apart in year 1. In study 3, there were 3 randomized treatment groups in year 1:2 doses of vaccine approximately 1 month apart, 1 dose of vaccine followed by 1 dose of placebo approximately 1 month later, and 2 doses of placebo approximately 1 month apart. In all 3 studies, subjects received a single dose of vaccine or placebo in year 2 [14]. The vaccines and placebos used in each study are described in Supplementary Text 1.

2.2. Nasal IgA evaluation

In all studies, nasal IgA and serum HAI antibody titers were evaluated in a subset of subjects enrolled. A separate population was defined each year. Nasal wash and serum samples were collected from subjects on 4 occasions over the 2 years: immediately before the first dose in year 1, approximately 1 month after the second dose in year 1, immediately before the year 2 dose, and approximately 1 month after the year 2 dose. In study 3, due to the randomization of subjects to 1 versus 2 doses of vaccine in the first year, additional samples were collected from subjects immediately before the second dose in year 1.

Nasal wash samples were tested by enzyme-linked immunosorbent assay (ELISA) for total IgA and strain-specific IgA antibody to the influenza A/H1N1, A/H3N2 and B vaccine strains. The primary endpoint for the IgA analysis was the ratio of influenza-specific IgA against A/H1N1, A/H3N2, or B strains in the vaccine to total IgA antibody. Geometric mean titers (GMTs) of absolute strain-specific IgA and total IgA were also evaluated at all time points. For strain-specific and total IgA, values for samples with no IgA were imputed as 50% of the minimum detectable value. Detailed methodologies and specific reagents used for this analysis are available in Supplementary Text 1. Serum antibody titers were evaluated by HAI assay using standard methods, as previously described [14,20]. Seronegative subjects were defined as those with a prevaccination HAI antibody titer of 4 or less; seropositive subjects were those with a titer greater than 4. An HAI response was defined as a 4-fold increase from prevaccination to postvaccination.

For descriptive purposes, the IgA response was categorized using 3 measurements: the percentages of subjects with ≥2-fold and ≥4-fold increases in the ratio of strain-specific to total IgA from baseline and the geometric mean fold rise (GMFR) in the ratio of strain-specific to total IgA from baseline. Results were evaluated separately for each study. The correlation between nasal IgA and serum HAI antibody responses was evaluated across studies for each influenza type/subtype.

To examine the relationship between IgA and the incidence of influenza illness, geometric mean postvaccination IgA ratios were compared between subjects with culture-confirmed influenza illness and those without evidence of culture-confirmed influenza illness. Influenza illness was evaluated for any influenza strain regardless of antigenic match to the vaccine as well as due to vaccine-matched strains. LAIV and placebo recipients were evaluated separately for each study. Additionally, given the small size of the immunogenicity cohorts in each study and the similarities in the design of the studies, a pooled analysis of all 3 studies was conducted to increase the statistical power to detect an effect. Only studies with at least 1 case of influenza illness were pooled.

Statistical comparison tests were conducted at the significance level of 0.05 using Fisher's exact test for the proportion of subjects with a ≥2-fold increase in titers and using the two-sample t-test for GMFRs and geometric means.

3. Results

3.1. Study subjects

In year 1, there were 183 (107 LAIV, 76 placebo), 101 (64 LAIV, 37 placebo), and 333 (226 LAIV, 107 placebo) subjects in studies 1, 2, and 3, respectively, with IgA data available for analysis. In year 2, there were 175 (94 LAIV, 81 placebo), 41 (24 LAIV, 17 placebo), and 791 (528 LAIV, 263 placebo) subjects in studies 1, 2, and 3, respectively. In each study, LAIV and placebo recipients were well-matched in regards to age and sex.

3.2. Increases in strain-specific IgA

Across the 3 studies, approximately one month after the second dose, a higher percentage of LAIV recipients had a ≥2-fold increase in strain-specific IgA ratio compared with placebo recipients (Fig. 1). In many comparisons, the difference between LAIV and placebo recipients was statistically significant. In study 3, responses were observed after a single dose but the differences compared to placebo recipients were more apparent after receipt of 2 doses of vaccine. Among subjects receiving only 1 dose of vaccine in year 1, a greater difference versus placebo was observed at the second versus first sample collection (approximately 2 months versus 1 month postvaccination). When the percentage of subjects with a ≥4-fold increase was evaluated, a similar pattern was observed, although response rates were lower. For LAIV and placebo recipients respectively, response rates were 26–39% versus 12–30% for

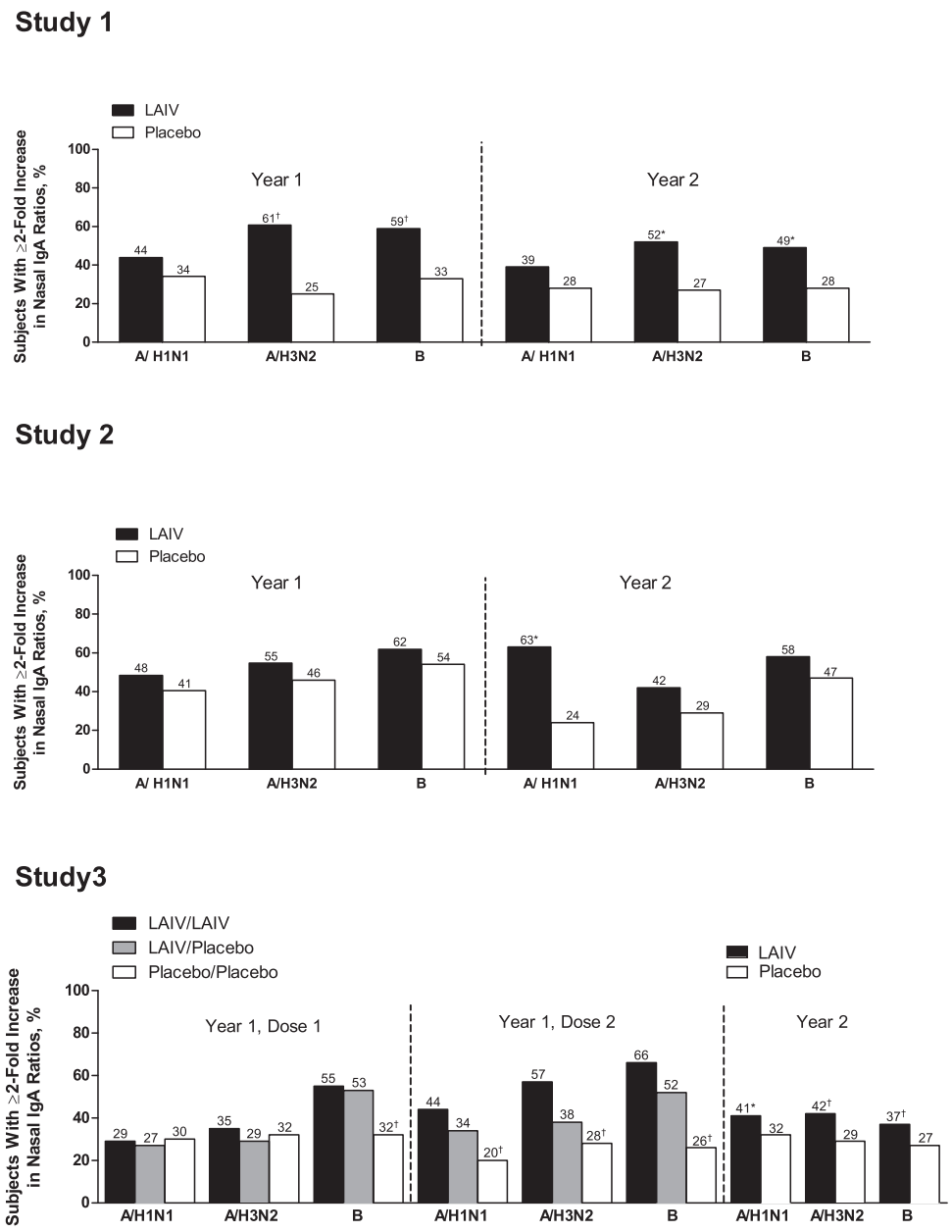

Fig. 1. Percentage of subjects with a ≥2-fold increase in strain-specific IgA ratios after vaccination with LAIV or placebo. *$P < 0.05$ vs placebo comparator, †$P < 0.01$ vs placebo comparator.

A/H1N1, 33–48% versus 20–27% for A/H3N2, and 46–59% versus 14–38% for B. When subjects were stratified by baseline serostatus, similar IgA responses were observed among seronegative and seropositive subjects.

Postvaccination GMFRs for strain-specific IgA ratios among LAIV recipients after 2 doses of vaccine in year 1 ranged from 1.4 to 6.2, compared to 0.5–2.0 among placebo recipients (Table 1). In year 2, GMFRs ranged from 1.2 to 4.6 among LAIV recipients and 0.8–2.2 among placebo recipients (Table 1). Postvaccination GMFRs in absolute strain-specific IgA, uncorrected for total IgA, trended higher than postvaccination GMFRs in strain-specific IgA ratios.

3.3. Increases in total IgA

Among LAIV and placebo recipients, total IgA increased from prevaccination to postvaccination by 1.0- to 2.4-fold in year 1 and 0.7- to 1.2-fold in year 2 (Table 2). Year 1 of study 3 was responsible for the greatest observed responses for LAIV and placebo recipients and 4 of the 5 statistically significant GMFRs. Because of the observed increases in total IgA from prevaccination to postvaccination in both placebo and vaccine recipients in year 1 of study 3, subject-level data by site were reviewed. In study 3, but not in studies 1 and 2, the total IgA content in year 1 prevaccination samples was lower among the initial subjects enrolled at sites and higher among subjects enrolled subsequently; linear regression analysis controlling for site showed that total IgA content in prevaccination samples increased significantly over calendar time in study 3 ($P = 0.002$).

3.4. Relationship between IgA and HAI responses

Across studies, data for both HAI and IgA responses following receipt of 2 doses was available for 392 LAIV recipients and 213 placebo recipients in year 1. Four-fold increases in HAI antibody

Table 1
Postvaccination GMFRs in ratios of strain-specific to total IgA for LAIV and placebo by study.

Strain	Study 1		Study 2		Study 3					
	Year 1, LAIV/LAIV (N = 107)	Year 1, Pbo/Pbo (N = 76)	Year 1, LAIV/LAIV (N = 64)	Year 1, Pbo/Pbo (N = 37)	Year 1, LAIV/LAIV		Year 1, LAIV/Pbo		Year 1, Pbo/Pbo	
					Dose 1 (N = 109)	Dose 2 (N = 113)	Dose 1 (N = 116)	Dose 2 (N = 112)	Dose 1 (N = 111)	Dose 2 (N = 107)
A/H1N1	1.4 (0.9, 2.2)	0.8 (0.5, 1.4)	2.1 (1.3, 3.4)	1.4 (0.7, 2.9)	0.9 (0.6, 1.3)	1.8 (1.2, 2.7)†	0.6 (0.4, 0.9)	0.8 (0.6, 1.3)	0.8 (0.5, 1.1)	0.6 (0.4, 0.8)
A/H3N2	3.6 (2.3, 5.5) †	0.9 (0.6, 1.4)	3.6 (2.1, 6.3)*	1.3 (0.7, 2.5)	1.1 (0.8, 1.6)	3.1 (2.0, 4.6)†	0.8 (0.5, 1.2)	0.9 (0.6, 1.5)	0.9 (0.6, 1.4)	0.8 (0.5, 1.2)
B	3.6 (2.2, 6.0) †	0.5 (0.3, 1.0)	6.2 (3.6, 10.8)*	2.0 (1.1, 3.9)	2.8 (1.8, 4.3)†	5.7 (3.8, 8.6)†	2.1 (1.3, 3.3)†	2.7 (1.6, 4.5)†	0.7 (0.5, 1.1)	0.6 (0.4, 0.9)

Strain	Study 1		Study 2		Study 3	
	Year 2, LAIV (N = 94)	Year 2, Pbo (N = 81)	Year 2, LAIV (N = 24)	Year 2, Pbo (N = 17)	Year 2, LAIV (N = 528)	Year 2, Pbo (N = 263)
A/H1N1	1.9 (1.3, 2.7)*	0.9 (0.6, 1.4)	2.4 (1.1, 5.5)	1.1 (0.5, 2.3)	1.4 (1.2, 1.7)†	0.9 (0.7, 1.1)
A/H3N2	2.5 (1.6, 4.0)†	1.0 (0.6, 1.5)	2.3 (1.2, 4.5)	1.2 (0.5, 3.1)	1.6 (1.4, 1.9) †	0.9 (0.7, 1.1)
B	2.5 (1.7, 3.8)†	1.1 (0.8, 1.5)	4.6 (1.6, 13.7)	2.2 (1.0, 5.1)	1.2 (1.0, 1.4)†	0.8 (0.6, 1.0)

LAIV = live attenuated influenza vaccine; pbo = placebo.
* $P < 0.05$, LAIV compared with placebo.
† $P < 0.01$, LAIV compared with placebo.

Table 2
Postvaccination GMFR in total IgA for LAIV and placebo by study.

Study 1		Study 2		Study 3					
Year 1, LAIV/LAIV (N = 107)	Year 1, Pbo/Pbo (N = 76)	Year 1, LAIV/LAIV (N = 64)	Year 1, Pbo/Pbo (N = 37)	Year 1, LAIV/LAIV		Year 1, LAIV/Pbo		Year 1, Pbo/Pbo	
				Dose 1 (N = 109)	Dose 2 (N = 113)	Dose 1 (N = 116)	Dose 2 (N = 112)	Dose 1 (N = 111)	Dose 2 (N = 107)
1.2 (0.8, 1.8)	1.9 (1.2, 3.0)	1.2 (0.9, 1.7)	1.0 (0.5, 1.8)	1.9 (1.3, 2.7)	1.4 (0.96, 2.1)	2.4 (1.6, 3.6)	2.0 (1.3, 2.9)	1.3 (0.9, 1.9)	1.6 (1.1, 2.4)

Study 1		Study 2		Study 3	
Year 2, LAIV (N = 94)	Year 2, Pbo (N = 81)	Year 2, LAIV (N = 24)	Year 2, Pbo (N = 17)	Year 2, LAIV (N = 528)	Year 2, Pbo (N = 263)
1.1 (0.9, 1.5)	1.0 (0.7, 1.4)	1.2 (0.7, 2.1)	0.7 (0.4, 1.5)	1.2 (0.98, 1.4)	1.2 (0.96, 1.5)

LAIV = live attenuated influenza vaccine; pbo = placebo.

titer for A/H1N1 were observed for 61% of LAIV recipients compared to 13% of placebo recipients ($P < 0.001$); for A/H3N2 and B, responses were 74% versus 16% ($P < 0.001$) and 76% versus 12% ($P < 0.001$) for LAIV versus placebo recipients, respectively. Among LAIV recipients, IgA responses were more frequently seen among subjects with an HAI response. Across studies, IgA responses to A/H1N1 were observed among 48% of subjects with a 4-fold HAI response, compared to 33% of those without a 4-fold HAI response ($P < 0.001$). For A/H3N2 and B, the proportions were 57% versus 37% ($P < 0.001$) and 65% versus 39% ($P < 0.001$), respectively. Among placebo recipients, IgA response rates were generally comparable for subjects with and without a HAI response: 22% versus 30% for A/H1N1 ($P = 0.5$), 41% versus 28% for A/H3N2 ($P = 0.2$), and 31% versus 34% for B ($P = 0.8$).

In year 2, 360 placebo recipients and 633 LAIV recipients had data for both HAI and IgA responses. For A/H1N1, A/H3N2 and B, HAI responses were 48% versus 16% ($P < 0.001$), 42% versus 16% ($P < 0.001$), and 29% versus 10% ($P < 0.001$) for LAIV versus placebo recipients, respectively. For LAIV recipients, IgA responses to A/H1N1, A/H3N2, and B were observed among 48% versus 35% ($P < 0.001$), 51% versus 38% ($P < 0.001$) and 48% versus 36% ($P < 0.001$) of those with and without a HAI response, respectively. As in year 1, IgA responses among placebo recipients were generally comparable for subjects with and without a HAI response: 21% versus 33% for A/H1N1 ($P = 0.1$), 26% versus 28% for A/H3N2 ($P = 0.9$), and 42% versus 27% for B ($P = 0.1$).

3.5. Relationship between igA and influenza illness

Based on pooled data from all 3 studies, in years 1 and 2, the mean postvaccination strain-specific to total IgA ratio was 3.1-fold higher ($P < 0.01$) and 2.0-fold higher ($P = 0.03$) among LAIV recipients with no culture-confirmed influenza illness compared with LAIV recipients who developed culture-confirmed influenza illness (Table 3). For each individual study and each type/subtype, mean postvaccination IgA ratios were generally higher among LAIV recipients with no evidence of influenza illness, although no individual comparison reached statistical significance.

When the analysis was restricted to culture-confirmed illness due to vaccine-matched strains, a 3.0-fold difference in IgA ratios between those with and without illness was still present among LAIV recipients in year 1 ($P = 0.02$). However, in year 2, there were very few subjects who developed vaccine-matched influenza illness ($N = 13$); the IgA ratio was 1.4-fold higher among those without influenza illness but this difference was not statistically significant ($P = 0.59$). In year 2 of study 3, there was a high incidence of influenza illness due to antigenically mismatched influenza B strains, due to significant circulation of viruses from the influenza B lineage not included in the vaccine; the B/Yamagata lineage strain B/Victoria/504/2000 was included in the vaccine but B/Hong Kong/1351/2002-like viruses of the B/Victoria lineage circulated. In year 2 of study 3, the mean IgA ratio against the vaccine-matched influenza B antigen was 1.8-fold higher among those subjects without illness compared with those with illness due to opposite lineage B strains ($P = 0.15$).

Among placebo recipients in years 1 and 2, there was no trend of higher IgA ratios among subjects without influenza illness (year 1 $N = 418$; year 2 $N = 498$) relative to those with influenza illness (year 1 $N = 34$; year 2 $N = 34$); no comparisons were statistically significant (Supplementary Table 1).

4. Discussion

This analysis of IgA responses from 3 clinical studies in young children confirms that LAIV induces measurable strain-specific IgA and demonstrates that these responses are associated with protection from subsequent influenza illness. IgA response rates were similar among subjects with and without prior exposure to influenza, as measured by baseline HAI antibody. For LAIV recipients, postvaccination strain-specific to total IgA ratios were consistently higher among those without influenza illness; thus higher amounts of strain-specific IgA appeared to protect the children from developing influenza illness. These findings are expected given that LAIV is a mucosal vaccine; however, they have not been previously demonstrated in large clinical studies.

The association between nasal strain-specific IgA and the incidence of influenza illness was consistently observed in years 1 and 2. The increased IgA response following 2 doses versus 1 dose of vaccine in study 3 also demonstrates that LAIV-induced mucosal antibody responses can be boosted with revaccination, consistent with data demonstrating enhanced clinical efficacy following revaccination [20]. However, the observed increases in IgA among LAIV recipients were of moderate magnitude and highly variable and substantial responses were observed among placebo recipients. This high variability is expected given that variation in nasal secretions and sample collection can lead to significant variability in sample volume and quality; this phenomenon explains the response rates observed among placebo recipients. As a result, the current data demonstrate that evaluations of strain-specific IgA responses in LAIV versus placebo recipients can provide a positive marker of vaccine-induced immunity but do not fully explain LAIV-induced protection from influenza illness.

A previous study by Boyce et al. demonstrated higher postvaccination IgA responses among pediatric LAIV recipients than the current analysis; IgA responses were observed in 62–85% of LAIV recipients compared to 0–33% of placebo recipients [27]. The higher response seen may be due to the small sample, more consistent sampling in a single study center, or slight differences in assay methodology. Additionally, Boyce et al. evaluated IgA an average of 82 days following vaccination, in contrast to the 56 days used in the studies presented here. Data from study 3 suggest that LAIV-induced strain-specific IgA responses continue to increase over time, as responses in subjects who received a single dose of LAIV were more apparent at 2 months versus 1 month after vaccination.

In adults vaccinated with LAIV, IgA responses have been less consistent and more modest than the responses observed in children. In previous exploratory studies conducted in adults, IgA response rates in LAIV recipients ranged from 10% to 40%, and in many cases, responses were not different from those observed among placebo recipients. However, 1 study reported that 80–100% of adult LAIV recipients achieved a $\geq$2-fold increase from baseline in strain-specific IgA antibodies [36]. However, the IgA analysis lacked a control group and thus it is difficult to interpret the high observed response.

Based on the detection of increased influenza-specific IgG and IgA circulating antibody-secreting B cells 1–2 weeks following LAIV vaccination with minimal subsequent increases in serum antibody and systemic memory B cells, Sasaki et al. proposed that LAIV provides protective immunity through a local B-cell memory response in the upper respiratory tract [26]. This mechanism is consistent with the current analysis and represents a plausible explanation of LAIV-induced antibody-mediated immunity, which is critical to block influenza virus infection [1]. However, it is clear that other aspects of the immune system contribute to LAIV-induced protection from influenza. In the current analysis and in a study by Boyce et al., the highest IgA responses were directed against the B strains followed by A/H3N2 [27]; however, LAIV has demonstrated similar and high efficacy in children against all 3 types/subtypes [11,37]. Studies have demonstrated that LAIV-induced immunity can also be partially explained by T-cell immunity [17,28,29,38] and serum antibody responses [39]. Stimulation of innate immunity

Table 3
Relationship between postvaccination ratio of strain-specific to total IgA and the incidence of culture-confirmed influenza by study and type/subtype: LAIV recipients.

Type/subtype	Study	Subjects without Influenza		Subjects with Influenza		*P*
		N	IgA ratio, GM	*N*	IgA ratio, GM	
Year 1						
A/H1N1	1	102	0.31	2	0.19	0.78
A/H3N2	1	102	0.54	3	0.16	0.24
A/H3N2	3	210	0.05	18	0.03	0.38
A/H3N2	Pooled	312	0.10	21	0.04	0.05
B	1	102	1.26	4	0.68	0.58
B	2	67	0.30	2	0.05	0.14
B	3	210	0.11	3	0.04	0.36
B	Pooled	379	0.26	9	0.15	0.46
Pooled	Pooled	379	0.18	32	0.06	<0.01
Year 2						
A/H1N1	3	374	0.05	4	0.01	0.06
A/H3N2	1	89	0.32	5	0.09	0.16
A/H3N2	2	23	0.74	1	0.77	0.99
A/H3N2	3	374	0.10	2	0.79	0.14
A/H3N2	Pooled	486	0.13	8	0.21	0.55
B	1	89	0.31	1	0.35	0.95
B	3	374	0.06	33	0.03	0.11
B	Pooled	463	0.08	34	0.04	0.03
Pooled	Pooled	486	0.09	45	0.04	0.03
Years 1 and 2 combined						
A/H1N1	Pooled	476	0.07	6	0.02	0.19
A/H3N2	Pooled	658	0.12	29	0.06	0.08
B	Pooled	702	0.14	43	0.05	<0.01
Pooled	Pooled	705	0.11	76	0.05	<0.01

GM = geometric mean.

via interferon and natural killer cells may also contribute to LAIV-induced protection, particularly when influenza circulates shortly after vaccination [38,40–42]. As an attenuated live virus vaccine, it would be expected that LAIV would induce a multi-faceted immune response, similar to that induced by wild-type influenza infection and other live virus vaccines [1]. It is likely that no single component of the response can fully explain the protective effect induced by LAIV.

Under the classification of correlates of protection for vaccination proposed by Plotkin [43,44], the association between LAIV-induced protection and measured IgA responses would be best classified as a relative co-correlate of protection. The relative co-correlate classification is appropriate because strain-specific IgA responses were associated with protection in LAIV recipients, but the level of response observed varied by strain and study and vaccine-induced protection has been shown to be correlated with other components of the immune response. Additionally, it is worth noting that no relationship between strain-specific IgA ratios and influenza illness incidence was observed among placebo recipients, which is a requirement for a more robust correlate of protection [43,44]. However, this lack of an association among placebo recipients is likely due to limited baseline strain-specific anti-influenza mucosal immunity among the study subjects given their young age.

Because of the high degree of variability observed, IgA would not be appropriate for evaluations requiring a precise quantitative assessment of the magnitude of LAIV-induced immune responses. In contrast, although they do not represent a correlate of protection, serum antibody levels following LAIV can be more consistently evaluated as the serum compartment is not subject to the same variability in content and sampling. For this reason, serum antibody responses following LAIV are the preferred method for evaluating the immunologic comparability of vaccine formulations or administration schemes [13,21,45–49]. In the current analysis, IgA and HAI responses were correlated, as IgA responses were more frequently observed among subjects with a HAI response.

The primary limitation of the current analysis is the small size of the study cohorts. Although the pooled sample enabled an examination of the relationship between IgA and the incidence of influenza illness, the analysis would have benefited from larger cohort populations. Averaging of IgA ratios across studies can also be problematic due to variability in values across types/subtypes and across studies. However, it is reassuring that the conclusions of the pooled analyses were supported by similar and consistent trends by study and type/subtype. In the analysis of the relationship between IgA and culture-confirmed influenza illness, it is possible that subjects without culture-confirmed influenza illness still experienced influenza infection; however, identification of these cases would likely have strengthened the observed relationship. Additionally, the assay was specific to IgA and did not evaluate nasal IgM or IgG antibody, which can also contribute to mucosal immunity [1]; a postvaccination increase in nasal wash IgG was observed in a prior study of LAIV [36].

In study 3, significant increases in total IgA were observed between baseline and postvaccination samples. Among prevaccination samples, which would not be subject to vaccine-induced effects, subjects who enrolled later had significantly higher total IgA, suggesting that site sample collection technique improved over time. This observation supports the practice of providing interspecimen standardization by reporting IgA values as ratios of specific to total IgA. A postvaccination rise in total IgA has also been reported following intranasal measles vaccination; however, the study lacked a placebo control and thus it was not possible to determine whether the total IgA increase was vaccine-attributable [50].

In conclusion, results from 3 clinical studies in young children demonstrated that LAIV induced measurable strain-specific IgA after vaccination and that IgA responses are associated with protection from subsequent influenza illness. However, the inherent heterogeneity in nasal antibody levels and variability in nasal specimen collection hinders the precise evaluation of mucosal antibody responses, and measured IgA responses do not fully explain LAIV-induced protection.

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Appendix A. Supplementary data

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