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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.

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