Comparisons of the Humoral and Cellular Immune Responses Induced by Live Attenuated Influenza Vaccine (LAIV) and Inactivated Influenza Vaccine (IIV) in Adults.

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ABSTRACT

Both live attenuated influenza vaccine (LAIV) and inactivated influenza vaccines (IIV) induce protective immunity against influenza. There is evidence that LAIV induces superior protection in children, whereas IIV may induce superior protection in adults. The immune mechanisms responsible for these differences have not been identified. We previously compared LAIV and IIV in young children 6-36 months of age, and demonstrated that while both induced similar hemagglutination-inhibiting (HAI) antibody responses, only LAIV induced significant increases in T cell responses. Here, 37 healthy adult subjects 18-49 years of age were randomized to receive seasonal influenza vaccination with LAIV or IIV. Influenza-specific HAI, T cell and secretory IgA (sIgA) responses were studied pre- and post-vaccination.

In contrast to responses seen in young children, LAIV induced only minimal increases in serum HAI responses in adults, significantly lower than responses induced by IIV. Both LAIV and IIV similarly induced only transient T cell responses in adults reactive with whole replication competent virus. In contrast, influenza-specific sIgA responses were more strongly induced by LAIV compared to IIV vaccine.

Our previous studies suggest that LAIV vaccine may be more protective than IIV in young children not previously exposed to influenza or influenza vaccines due to increased vaccine-induced T cell and/or secretory IgA responses. Our current work suggests that in adults with extensive and partially cross-reactive pre-existing influenza immunity, LAIV boosting of sIgA responses reactive with hemagglutinin (HA) and non-HA antigenic targets expressed by circulating influenza strains may be an important additional mechanism of vaccine-induced immunity.
INTRODUCTION

Seasonal influenza vaccinations are recommended in the United States for all persons ≥6 months old (1). Two types of influenza vaccines, live attenuated influenza vaccine (LAIV) and inactivated influenza vaccine (IIV), formulated with circulating type A H1N1, H3N2 and type B antigens, are widely used (LAIV in persons 2-49 years of age; IIV in persons ≥6 months of age) and have been shown to be safe and effective (2, 3). IIV, administered intramuscularly, contains inactivated viral components, primarily purified hemagglutinin (HA) antigens. LAIV, administered intranasally, contains a full complement of viral components. LAIV replicates within epithelial cells in the nasopharynx, mimicking natural viral infection. Because of the mucosal administration route and production of viral antigens within host antigen presenting cells, LAIV vaccination could be more effective than IIV in inducing mucosal responses and/or overall T cell responses. On the other hand, pre-existing cross-reactive influenza immunity could limit LAIV replication and therefore limit LAIV immunogenicity.

Previous phase III clinical trials have demonstrated that both LAIV and IIV can induce significant protection in persons aged 2 to 50 years (4). IIV is licensed and protective (although somewhat less so) for young children aged 6-24 months and adults over 50 years old. Three randomized, controlled efficacy trials in children >6 months and ≤18 years old consistently demonstrated that LAIV was significantly more protective than IIV (4-7). Similarly, in adults older than 16 years, some comparative trials showed that LAIV was at least as effective as IIV (8-10). However, 2 adult trials showed that IIV was more protective than LAIV (11-13), and
thus there is some controversy over whether LAIV can be as effective as a well-matched IIV seasonal vaccine in adults with previously extensive influenza exposure (2, 14).

Several factors are likely to impact the relative efficacy of LAIV and IIV in different populations: route of administration, live vs inactivated antigens administered, intrinsic immunogenicity of the vaccines, pre-existing immunity and immune status of the vaccinee. Host status can have major effects on vaccine-induced responses. For example, pre-existing immunity can inhibit the response to a vaccine that must infect and replicate for optimal immunogenicity (15, 16). Furthermore, relative immunodeficiencies can limit optimal vaccine-induced responses, and may have a greater effect on reducing responses to IIV vaccines with less natural adjuvant properties. We have previously shown that compared with IIV, LAIV induced similar serum HAI responses but significantly better T cell responses in children 6-36 months of age (17). In the current study, we evaluated 3 different influenza-specific immune responses induced in adult subjects given LAIV or IIV: serum antibody responses by HAI, T cell responses by ELISPOT and ICS, and sIgA responses by ELISA. The combination of all three of these immune responses using state of art assays have not been studied in the same individuals before, which is the strength of this study. We now report in adults that: 1) serum HAI responses induced by IIV were significantly higher than serum HAI responses induced by LAIV, 2) LAIV and IIV induced comparable but only transient T cell responses, and 3) LAIV was more effective than IIV in inducing secretory IgA responses. We hypothesize that LAIV has more intrinsic immunogenicity than IIV, and when pre-existing heterotopic immunity does not reduce this immunogenicity, LAIV can induce better T cell responses, promote similar serum HAI titers, and uniquely induce mucosal immune responses relevant for protective immunity. Furthermore, even
in the absence of detectable increases in serum HAI, LAIV vaccination in adults may enhance protection by increasing influenza-specific secretory IgA responses.

MATERIALS AND METHODS

Subjects and vaccines. Thirty-seven healthy adults 18-49 years of age, without symptoms of upper respiratory illness, were recruited to participate in this study. After informed consent was obtained, subjects were randomized 1:1 to receive a single dose of either trivalent LAIV known commercially as FluMist®, MedImmune, or trivalent IIV, known commercially as Fluzone®, Sanofi Pasteur. Both vaccines were licensed 2010-2011 trivalent seasonal products. LAIV (FluMist®), a 0.2ml (intranasal) dose formulated to contain 10^{6.5-7.5} FFU (fluorescent focus units) of live attenuated influenza virus reassortants of each of three strains: A/California/7/2009 (H1N1), A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008. IIV (Fluzone®), a 0.5 ml (intramuscular) dose of inactivated influenza vaccine contained 15 µg of each seasonal viral type for a total 45µg dose. Strains used: A/California/07/2009, x-179A (H1N1), A/Victoria/210/2009, x-187 (an A/Perth/16/2009-like virus) (H3N2), and B/Brisbane/60/2008. Upon enrollment, a single dose of seasonal LAIV or IIV was administered to each study volunteer. Serum, peripheral blood mononuclear cells (PBMC) and nasal wash (NW) specimens were obtained on day 0 (pre-vaccination) and on days 7 and 45-51 (hereafter day 45) post-vaccination.

Safety. Since the study involved administration of licensed vaccines indicated for the population enrolled, detailed safety data were not collected. However, study subjects had 3
clinic visits, and 2 follow-up telephone contacts, during which information on health status and any adverse events were solicited and documented.

Serum Hemagglutination Inhibition (HAI) tests were performed as previously described (18). Serum samples, obtained pre-vaccination, and 7 days and 45 days post-vaccination were tested in duplicate against season-matched influenza HAI test antigens obtained from the CDC. Test antigens were beta-propiolactone-inactivated influenza strains H1N1 (A/California/7/2009), H3N2 (A/Perth/16/2009), and B (B/Brisbane/60/2008).

Cell Mediated Immunity (CMI) ELISPOT assays detecting numbers of IFN-γ producing cells were performed with PBMCs obtained pre-vaccination and 7 and 45 days post-vaccination, using methods previously described (17). PBMCs were stimulated in triplicate with medium alone, a live heterotypic influenza virus (A/H3N2/California/07/2004), Influenza A peptide pool I (a pool of 35 class I peptides) and Influenza A peptide pool II (a pool of 16 class II peptides) combined, or FluMist® (2010-2011 formulation).

Antigen-specific proliferation and production of IFN-γ by T cells were measured using a 7 day CFSE-dilution, intracellular cytokine staining (ICS) assay previously described (17). CFSE-stained PBMCs were stimulated with live influenza virus (A/H3N2/California/07/2004), Influenza A peptide pool I (pool of 35 class I peptides), Influenza A peptide pool II (pool of 16 class II peptides), Influenza A peptide pools I & II combined, and FluMist® (2010-2011 formulation), or rested in medium alone, for one week at 37°C with 5% CO₂. IL-2 was added to a concentration of 20 U/ml on the 4th day of incubation. Absolute numbers of CD4+ and CD8+
T cells that were CFSE-low and IFN-γ+ were determined by multiplying viable cell counts on day 7 by percentages of each T-cell subset.

Secretory IgA antibodies to Hemagglutinin (HA) from Influenza A/H1N1, Influenza A/H3N2, and Influenza B were evaluated by ELISA of nasal wash specimens obtained pre- and 7 and 45 days post-vaccination. Microtiter plates were coated overnight with strain-matched, full-length glycosylated recombinant influenza HA proteins (obtained from Sino Biological, Inc., Beijing, P.R. China) Influenza H1N1 HA (A/California/07/2009), Influenza H3N2 HA (A/Perth/16/2009), and Influenza B HA (B/Brisbane/60/2008) at 1µg/ml. Nasal wash specimens, which had been sonicated and concentrated 5-fold, were serially diluted in incubation buffer (PBS/0.1% Tween-20/1% BSA) starting at 1:2.5 dilutions, and added to the washed plates together with controls. After overnight incubation, the plates were washed and biotin-conjugated affinity purified goat anti-human IgA (KPL #16-10-01), was added at 1:4,000. Plates were incubated for 2 hours at 37°C, followed by a wash step and the addition of Avidin-Alkaline phosphatase (KPL #15-30-00) at 1:4,000. Following incubation, plates were washed and pNPP substrate was added, and absorbance measured by spectrophotometer 1 hour later. After background subtraction, linear regression plots were generated (log absorbance vs log dilution) and endpoint titers (EPT) were determined using a 0.2 O.D. cut-off.

Statistical methods. Group antibody data were expressed as geometric mean titers. Mean baseline responses plus 2 standard errors were used to define cut-offs for positive responses. Transformed continuous data were compared by ANOVA. Non-transformed continuous data
were compared by Wilcoxon matched pairs tests (pre- to post-vaccination comparisons) or
Mann-Whitney U test (group comparisons at individual time points). Dichotomous responses
were compared with 2-sided Fisher exact tests. Outliers for sIgA were defined as having pre-
vaccination nasal wash sIgA titers that were greater than the mean of all pre-vaccination levels
plus 1 standard error.

RESULTS

Study Subjects. Enrollment included 19 LAIV and 18 IIV recipients. Pre- and post-
vaccination samples were collected from 18 LAIV and 18 IIV recipients. No serious adverse
events occurred during the 6 month follow up period. One subject in the LAIV group did not
return for follow-up.

Serum HAI responses. Shown in Table 1 are the serum HAI Geometric Mean Titer
(GMT) responses detected in adults following IIV or LAIV vaccination. Presented are results
for each group at 3 different time points pre- (day 0) to post-vaccination (days 7 and 45) using
each of the 3 HA antigens present in the vaccine strains. A major difference was seen in
vaccine-induced HAI responses comparing IIV and LAIV groups. In the IIV group, the HAI
GMT progressively increased pre-to post-vaccination, achieving levels 5-10 fold higher than
baseline. In fact, the confidence intervals for IIV group pre-vaccination responses did not
overlap with the confidence intervals for the Post-vaccination #2 visit responses with any of the
3 HAI assay targets (H1, H3 or B HA). In contrast, HAI responses in the LAIV group were
similar pre- to post-vaccination. GMT HAI responses were significantly different between IIV
and LAIV responses at both post-vaccination time points. These results were not due to
differences in baseline HAI; none of the pre-vaccination comparisons between IIV and LAIV HAI responses showed significant differences.

IFN-γ ELISPOT responses. Overall influenza-specific T cell responses detected by IFN-γ ELISPOT assay are shown in Figure 1. As anticipated, both groups of volunteers had significant baseline responses reactive with the conserved peptide pools, the heterotypic H3N2 virulent virus and the LAIV components, consistent with previous priming due to previous influenza infections and/or vaccinations. Responses to FluMist® components were significantly increased on day 7 post-vaccination in both LAIV and IIV vaccination groups, and LAIV induced a significant response to live heterologous H3N2 influenza on day 7 post-vaccination. Both LAIV and IIV were able to boost, at least transiently, T cell responses in adults to live H3N2, and to FluMist®.

Flow based CD4+ and CD8+ T cell proliferative and IFN-γ responses. Flow-based, intracellular staining assays were used to detect memory/effector T cells capable of both proliferating and producing the effector cytokine IFN-γ, as well as identification of the T cell subsets producing antigen-specific responses. Similar to the IFN-γ ELISPOT studies, CD4+ T cell responses increased after vaccination with both LAIV and IIV vaccines. Figure 2 shows that CD4+ T cells reactive with a previously circulating heterotypic H3N2 influenza strain, as well as with the matched LAIV components, were increased in both vaccine groups at day 7 post-vaccination. However, CD4+ T cell responses induced by the conserved influenza peptide pools and CD8+ T cell responses induced by FluMist®, heterotypic H3N2 virus and conserved influenza peptide pools were not significantly increased post-vaccination in either group (data...
not shown). In general, the vaccine-induced CD4+ T cell responses were short-lived, falling to baseline levels by day 45.

**Secretory IgA responses.** To compare mucosal immune responses induced by the 2 vaccines in adults, we measured influenza strain-specific nasal wash secretory IgA by ELISA. The most significant results were seen at day 45 post-LAIV. Table 2 presents the medians and ranges of sIgA titers for each vaccine group pre-vaccination and day 45 post-vaccination. The median titers for Influenza H1, H3 and B HA-specific antibody responses modestly but significantly increased in LAIV recipients, with baseline medians ranging from 9.75-32.25 and post-vaccination medians from 16.75 to 57.25. In contrast, IIV vaccine group sIgA HA binding titers 45 days after vaccine were similar to pre-vaccine levels. Figure 3 presents pre- to post-vaccination mean fold-increases in sIgA detected at 7 days post vaccine by vaccine group, and the proportions of subjects in each group mounting a 4-fold or greater increase above baseline levels. Mean fold increases for all 3 HA components were 8.4-9.5-fold in LAIV recipients, compared to 1.7-2.3-fold in IIV recipients. In general, LAIV recipients had higher average sIgA fold-increases over IIV and had significantly higher frequencies of 4-fold or greater sIgA responses than IIV recipients for 2 of 3 vaccine strains. Higher sIgA responses persisted at day 45 post-vaccine in the LAIV group, but not the IIV group.

**DISCUSSION**

We compared serum antibody, T cell, and secretory IgA immune responses in normal healthy adults, aged 18-49 years, who received either IIV or LAIV seasonal flu vaccines. Each of these responses have been measured in previous vaccine trials comparing IIV and LAIV (14, 19-22),
but the comparisons have not all been studied in the same group of adults. The seasonal vaccines administered contained antigens from equivalent influenza strains, but were either inactivated and administered by IM route, or live, attenuated and administered by mucosal route. Only subjects who received IIV had significant and sustained HAI responses to matched seasonal Influenza A/H1N1, Influenza A/H3N2, and Influenza B viruses, whereas subjects who received LAIV had modest to no increase in HAI titer to any of the influenza antigens. Both LAIV and IIV induced similar, if transient increases in influenza-specific memory/effector T cell responses to both seasonally matched influenza components and to a live, previously circulating heterotypic strain of Influenza A/H3N2. Some of these T cell increases persisted for over 6 weeks. Boosted T cell responses included responses to highly conserved class I and class II Influenza peptide epitopes relevant for induction of universal influenza immunity. Subjects who received LAIV were more likely to respond with 4-fold or greater increases one week following vaccination, and sIgA increases induced by LAIV were more likely to persist compared to IIV. The humoral and T cell responses reported here in adults are in contrast with previous results reported in young children (17), where both IIV and LAIV induced similar humoral immune responses, but only vaccine regimens including LAIV induced influenza-specific CD4+, CD8+ and gamma delta T cell responses important for cell-mediated immune protection.

It is interesting that IIV recipients developed serum HAI responses but not sIgA responses, while LAIV recipients developed sIgA responses but not serum HAI responses. Possible explanations for the apparent dissociation of mucosal and systemic antibody responses are the differences in vaccine formulations and the mode of vaccine delivery. Purified influenza protein antigens administered parenterally induce B and T cells with systemic homing molecules (e.g. cutaneous lymphatic antigen), important for recognition of molecular structures lining...
endothelial cells required for transpedesis into peripheral cutaneous tissues. Whereas live
attenuated whole viruses in LAIV presented intranasally provide for replication and prolonged
antigen stimulation in the upper respiratory tract, increasing inflammation and stimulation in the
mucosa. Mucosal stimulation induces B cells that express mucosal homing molecules that are
then upregulated, enhancing trafficking of memory immune T and B cells to the mucosa. For
example, the α4β7 integrin complex is upregulated on the surface of lymphocytes activated in the
Peyer’s patches. This integrin specifically binds to MadCAM1 on endothelial cells and triggers
transendothelial migration from the vasculature into peripheral mucosal tissues (23, 24). LAIV
induces B cells with mucosal trafficking/respiratory tract resident cells. Circulating T cells could
be differentially induced by LAIV to facilitate mucosal trafficking in vaccine-induced B cells
activated by vaccination; and increased mucosal resident T cells may directly recruit B cells to
the upper respiratory tract via specific chemo attractants (23). In addition, it would be interesting
in future studies to measure CCR5 and CCR7 expression on T cells induced by LAIV vs IIV
because these chemokine receptors have also been reported to facilitate lung trafficking of
vaccine-induced T cells (25, 26).

The adult response to LAIV and IIV reported here are in contrast to those previously reported
in children. In addition to the striking differences in HAI responses between the two age groups,
the T cell increases detected in this adult study were much less impressive than the LAIV-
induced T cell responses detectable in children 6-36 months old (17). These differences may be
due in part to pre-existing immunity present in most adults due to multiple prior exposures to live
circulating influenza strains and to previous influenza vaccinations. In a recent report Barria et
al (15), evaluated HAI responses to H1N1 HA with respect to pre-existing antibody titers and
noted that the small subset of adults achieving a 4-fold or higher HAI response to HIN1 HA
following LAIV vaccine had low to negative pre-vaccination HAI titers. The relative
immunological naiveté of young children allows more prolonged replication of LAIV in the
upper respiratory tract, which may result in better stimulation of multiple T and B cell subsets.
Inclusion of pathogen-associated molecular patterns, (PAMPs) recognized by cells of the innate
immune system, facilitates robust immune responses in naïve LAIV recipients. Conversely,
adults with extensive cross-reactive influenza immunity substantially reduce “take” or duration
of replication of LAIV, thus preventing the stimulation of new systemic immune responses. IIV
may induce only new B cell, new CD4+ T cell responses, and booster responses in
memory/effector cells, while LAIV can induce new B cell, new CD4+ T cell, new CD8+ T cell
and booster responses in both B and T memory/effector cells.

Using the ferret influenza intranasal challenge model, Cheng et al (27), evaluated protective
responses elicited by LAIV or IIV following upper respiratory challenge of seropositive ferrets.
While both vaccines elicited humoral responses in the ferrets, only LAIV provided protection
from respiratory challenge with a live heterologous influenza virus. Potential mechanisms of
sIgA protection may include blocking of viral host cell attachment at the site of initial mucosal
infection, intracellular uptake of sIgA and blocking of viral un-coating, or redirection for uptake
of influenza particles by respiratory macrophages or other phagocytes which can mediate
intracellular killing and inhibition of influenza replication (28). These protective mechanisms
afforded by LAIV may explain the beneficial effects of LAIV vaccination in adults despite
absence of serum HAI responses.

Future studies should include the identification of epitopes most important in eliciting
protective sIgA responses, including HA, HA stalk region and other viral antigens that might
confer cross-protective sIgA responses. In vitro protection assays to directly assess sIgA-
mediated inhibition of viral replication in human macrophages would facilitate understanding of sIgA protective capacity on a larger scale than is possible with the animal model. Studies are also needed to understand the differences and clinical importance of sIgA in conferring protective immunity in children and adults, and the role of pre-existing immunity in these populations. Intranasal vaccination with an inactivated whole influenza virus has been shown to induce HAI and neutralizing antibody responses in nasal secretions (29). Future studies should include evaluation of functional antibodies elicited by LAIV and IIV in nasal wash specimens in adults and children, and to look for correlations between sIgA and vaccine efficacy in these populations.

Previous studies suggest that LAIV vaccine may be more protective than IIV in young children who have not previously been vaccinated or exposed to influenza, due to either enhanced T cell or sIgA responses (17). However, in adults with extensive pre-existing influenza immunity, LAIV boosting of influenza-specific sIgA responses may be an important additional mechanism of vaccine-induced immunity. Influenza-specific sIgA and T cell responses may be important correlates of protection against heterotypic and emerging influenza strains, and should be investigated as targets for next generation influenza vaccines.

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REFERENCES


Figure 1. Overall T cell responses induced by LAIV and IIV in healthy adults. PBMC from day 0 (pre-vaccination), day 7 and day 45 post-vaccination were incubated overnight in IFN-γ ELISPOT wells with medium alone, a highly conserved influenza-specific peptide pool which contains CD4 and CD8 T cell epitopes predicted to cover all populations by 200% (PP-I+II; (17), a live infectious 2004 H3N2 seasonal influenza strain (Live H3N2), and the matching FluMist components used to vaccinate the LAIV group (FluMist). Numbers of IFN-γ spot-forming cells (SFC) per million PBMC were calculated. Similar, but transient increases in overall influenza-specific T cells were seen in both the LAIV and TIV vaccination groups. Note that responses directed against the conserved influenza-specific predicted T cell epitopes were increased at baseline and increased only marginally post-vaccination. Whiskers are mean +/- standard error, * p <.05, by Wilcoxon’s Matched Pairs, **p <.05, by Repeated Measures ANOVA with Tukey post hoc, for differences within groups from baseline. N (IIV group) = 18 all visits, N (LAIV group) =19, 18, 17, visit 1, 2, & 3.

Figure 2. CD4+ memory/effector T cells Reactive with Heterotypic H3N2 and All Components of LAIV. PBMC from days 0 (pre-vaccination), day 7 and day 45 post-vaccination were thawed and labeled with CSFE. These CFSE-labeled PBMC were incubated for 1 week with medium alone, a live infectious 2004 H3N2 seasonal influenza strain (Live H3N2), and the matching FluMist components used to vaccinate the LAIV group (FluMist). After the 1 week stimulation cultures were complete, T cells were harvested, stained with surface markers and then intracellularly for IFN-γ. Absolute numbers of CFSE low and IFN-γ+ CD4+ T
20 cells (AN, absolute numbers calculated by multiplying T cell subset FACS percentages by total
viable cells recovered) are shown. These results are similar to the IFN-γ ELISPOT results seen
in Figure 1. Both LAIV and IIV induced similar, but transient increases in CD4+ T cells.

*p<.05, comparing post-vaccination with pre-vaccination responses by Wilcoxon matched pairs
test for differences within groups from baseline. N (IIV group) = 18 all visits, N (LAIV group)
=19, 18, 17, visit 1, 2, & 3, consecutively.

Figure 3. Fold-Changes in HA-specific Nasal Wash sIgA Endpoint Titers 7 days Post-
vaccination. Nasal wash specimens were collected pre-vaccination, and 7 days and 45 days
post-vaccination. Endpoint titers were determined for each sample incubated in ELISA wells
coated with H1N1 HA, H3N2 HA, and FluB HA matching the 3 influenza components of the
LAIV and IIV vaccines. Shown are average fold changes from baseline at day 7 post-
vaccination for all 3 HA antigens. Shown in parentheses is the proportion of each group
mounting a 4-fold or greater increase from baseline. *P <.05 by Fisher’s Exact Test, comparing
LAIV and IIV day 7 post-vaccination responses.
### Table 1. Comparison of Serum Hemagglutination Inhibition Antibody Responses

<table>
<thead>
<tr>
<th>HAI Assay Target</th>
<th>HIV (N=18)</th>
<th>LAIV (N=18)</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Influenza A/H1N1</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre-Vaccination</td>
<td>11.76 (6.2,22.4)</td>
<td>11.11 (6.3,19.9)</td>
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<tr>
<td>Post Vaccination, Day 7</td>
<td>47.03 (24.3,91.1)</td>
<td>9.33 (5.4,16.3)</td>
<td>&lt;.001</td>
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<tr>
<td>Post Vaccination, Day 45</td>
<td>101.59 (51.6,200)</td>
<td>12.70 (7.0,23.2)</td>
<td>&lt;.0001</td>
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<tr>
<td><strong>Influenza A/H3N2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Vaccination</td>
<td>6.35 (4.1,9.9)</td>
<td>7.44 (4.6,12.0)</td>
<td>0.63</td>
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<tr>
<td>Post Vaccination, Day 7</td>
<td>17.96 (10.5,30.8)</td>
<td>7.13 (4.1,11.7)</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>Post Vaccination, Day 45</td>
<td>32.00 (17.5,58.4)</td>
<td>9.70 (5.9,16.0)</td>
<td>&lt;.01</td>
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<tr>
<td><strong>Influenza B</strong></td>
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<tr>
<td>Pre-Vaccination</td>
<td>7.41 (5.2,10.6)</td>
<td>10.71 (6.6,17.5)</td>
<td>0.25</td>
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<tr>
<td>Post Vaccination, Day 7</td>
<td>21.77 (14.2,33.4)</td>
<td>11.31 (6.8,18.77)</td>
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<tr>
<td>Post Vaccination, Day 45</td>
<td>40.32 (24.4,66.7)</td>
<td>12.70 (7.9,20.5)</td>
<td>&lt;.01</td>
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**Note.** The P value of comparisons among treatment groups in hemagglutination inhibition (HAI) geometric mean titers (GMTs) are calculated by t-test. CI, confidence interval.
Table 2. Comparison of Nasal Wash Hemagglutinin-specific sIgA Responses

<table>
<thead>
<tr>
<th>ELISA Assay Target antigen</th>
<th>Vaccine Group</th>
<th>Median NW (Range)</th>
<th>Median NW (Range)</th>
<th>*P-value Pre- versus Day 45 Post-vaccine</th>
</tr>
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<tbody>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Influenza A/H1N1 HA</td>
<td>IIV</td>
<td>18</td>
<td>20.75 (1.25-2328)</td>
<td>0.717</td>
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<tr>
<td></td>
<td>LAIV</td>
<td>16.25 (3-41)</td>
<td>28.5 (4-197)</td>
<td>0.05</td>
</tr>
<tr>
<td>Influenza A/H3N2 HA</td>
<td>IIV</td>
<td>26.5 (5-69)</td>
<td>31.5 (3-476)</td>
<td>0.064</td>
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<tr>
<td></td>
<td>LAIV</td>
<td>32.25 (1.25-88)</td>
<td>57.25 (10-458)</td>
<td>0.004</td>
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<tr>
<td>Influenza B HA</td>
<td>IIV</td>
<td>16</td>
<td>16.5 (3-50)</td>
<td>0.754</td>
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<tr>
<td></td>
<td>LAIV</td>
<td>9.75 (3-47)</td>
<td>16.75 (5-113)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Note. Shown are median sIgA anti-HA endpoint titers and ranges. *P by Wilcoxon’s Matched Pairs, Day 0 (pre-vaccination) outliers (1SE) excluded.
Figure 1. IFN-γ SFC/million PBMC

- Day 0
- Day 7
- Day 45

IIV Group
LAIV Group

- Medium
- PPI + PPII
- Live H3N2
- FluMist

* *
Figure 2. Absolute Number CD4+/CFSE\textsuperscript{lo}/IFN-\gamma+ T Cells

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<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIV Group</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LAIV Group</td>
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</tr>
</tbody>
</table>

Medium Live H3N2 FluMist
Figure 3.

Post-vaccination NW sIgA Fold-Increases at Day 7 by Vaccine Group
Mean; Whisker: Mean±SE

- Fold Increase IgA anti-H1N1 HA at Day 7
- Fold Increase IgA anti-H3N2 HA at Day 7
- Fold Increase IgA anti-FluB HA at Day 7

(Proportion ≥ϰ-fold Increase at Day 7)
*p<0.05 by Fisher’s Exact Test

- IIV
- LAIV

(3/18) (0/16) (1/16) (5/18) (7/18)