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Comparative analysis of influenza A(H3N2) virus hemagglutinin specific IgG subclass and IgA responses in children and adults after influenza vaccination

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ABSTRACT

Two different influenza vaccines are generally used in many countries; trivalent live attenuated influenza vaccine (LAIV3) and trivalent inactivated influenza vaccine (IIV3). Studies comparing the antibody response to IIV3 and LAIV3 commonly investigate the seroprotective response by hemagglutination-inhibition (HI) assay. However, there is limited data regarding comparative analysis of IgG subclass and IgA responses induced by LAIV3 and IIV3.

Fifteen children <5 years received 2 doses of LAIV3 while 14 children aged 10–17 years received one dose. In addition, 15 adults were vaccinated with either intranasal LAIV3 or intramuscular IIV3. We analyzed the H3N2 humoral responses by HI assay and the hemagglutinin (HA) specific IgG1, IgG2, IgG3, IgG4 and IgA1 responses by ELISA. Furthermore, we investigated the avidity of induced IgG antibodies.

Pre-existing seroprotective HI antibodies were present in adults (73%) previously vaccinated with IIV3. Vaccination resulted in a significant increase in HI titers in all groups, except LAIV3 vaccinated adults. Furthermore, a negative correlation between age and HI titers in LAIV3 vaccinated subjects was observed post-vaccination. LAIV3 in children and IIV3 in adults induced HA-specific IgG1, low IgG3 but no IgG2 or IgG4. Moreover, significant IgA1 responses were only induced in children. Interestingly, IIV3 and LAIV3 induced IgG antibodies with comparable and significantly augmented avidity post-vaccination in children and adults.

Our results suggest that age and/or exposure history play a significant role in determining the antibody response.

Clinical trial registry: ClinicalTrials.gov NCT01003288 and NCT01866540

1. Introduction

Influenza is one of the most common respiratory infections representing a cause of concern in the field of public health [1]. Each year, seasonal influenza infection can cause up to 5 million severe cases and between 250,000 and 500,000 deaths worldwide [2,3]. Vaccination remains the most effective preventative measure against infection and limits morbidity and mortality caused by influenza. The effectiveness of influenza vaccination varies in different age groups and by vaccine formulations [4–6].

Currently, there are two main types of seasonal influenza vaccines: the trivalent inactivated influenza vaccine (IIV3) and trivalent live attenuated influenza vaccine (LAIV3). Although recently quadrivalent vaccines containing two B strain lineages have become available, namely LAIV4 and IIV4. Studies that have investigated the antibody responses after IIV3 and LAIV3 vaccination have focused on the classic serological assays, such as hemagglutination-inhibition assay (HI) and microneutralization assay (MN) [7–10]. These antibody responses are mainly directed

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to the major viral surface glycoprotein, hemagglutinin (HA). HA has important functions essential for infection, such as recognition of host cells' receptors and fusion of viral and endosomal membranes. Antibodies to HA are measured by classical serology as surrogate correlates of protection. However, there are no established correlates of protection for LAIV. Furthermore, there is limited data documenting the differences in systemic IgG and IgA subclass responses after vaccination in children and adults.

IgG levels are important for influenza vaccination responses and protection [11,12]. The four subclasses of IgG in humans; IgG1, IgG2, IgG3 and IgG4, differ in function [13]. In particular, IgG1 and IgG3 are involved in many important immunological functions, including complement fixation, opsonization as well as virus neutralization [6]. Two IgA subclasses, IgA1 and IgA2 are involved in the protection in the local mucosa, including the upper respiratory tract where the influenza virus causes infection [14,15].

We conducted this study to investigate the differences in HA-specific IgG subclass and IgA antibody responses induced by LAIV3 in children and adults and by IIV3 in adults. We also evaluated the quality of the induced IgG antibodies.

2. Materials and methods

2.1. Study design

All participants >12 years and parents provided written informed consent before inclusion in the study, which had ethical and regulatory approval (ClinicalTrials.gov NCT01003288 and NCT01866540). All individuals were vaccinated during the winters of 2012 and 2013. Fifteen healthy children <5 years of age received two doses of LAIV3, 28 days apart, while 14 healthy children aged 10–17 years old received 1 dose of LAIV as recommended by the manufacturer. Fifteen healthy adults received a single dose of LAIV3. As a comparator control, an additional 15 adults were vaccinated with IIV3. All IIV3-vaccinated adults were healthcare workers and had received prior seasonal influenza vaccination, as well as pandemic H1N1 vaccine in 2009 (Table 1).

The LAIV3 (Fluenz™, AstraZeneca, UK) for 2012–2013 contained $10^{7.0\pm0.5}$ FFU for each of A/California/7/2009(H1N1)pdm09, A/Victoria/361/2011(H3N2), and B/Wisconsin/1/2010. The 2013–2014 LAIV3 vaccine contained A/California/7/2009 (H1N1)pdm09, A/Victoria/361/2011 - H3N2-like strain (A/Texas/50/2012) and B/Massachusetts/02/2012. The IIV3 (split-vaccine) (Vaxigrip®, Sanofi Pasteur, France) containing 15 μg HA of A/California/07/2009-like virus (H1N1)pdm09, A/Texas/50/2012 (H3N2) and B/Massachusetts/02/2012. Serum samples were collected prior to vaccination, and after vaccination (Fig. 1). All serum samples were aliquoted and stored at −80 °C before use.

2.2. Hemagglutination-inhibition assay (HI)

Serum samples were treated with receptor destroying enzyme and run in the HI assay using the homologous H3N2 vaccine strain as previously described [16]. Seroprotection was defined as an HI titer ⩾ 40. HI titers < 10 were assigned a value of 5 for calculation purposes.

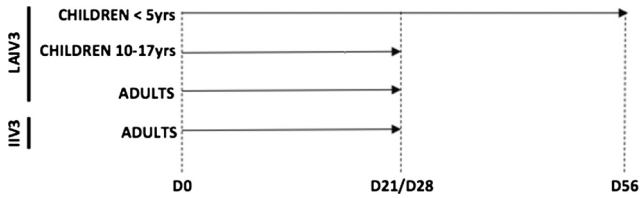

Fig. 1. Study design. Fifteen healthy children <5 years old, 14 children aged 10–17 and 15 adults were vaccinated with Live Attenuated Influenza Vaccine (LAIV3). In addition, 15 adults were vaccinated with Trivalent Inactivated Influenza Vaccine (IIV3) as a control. Children <5 received 2 vaccine doses, 28 days apart, while the remaining participants received 1 dose. Plasma was collected at day of vaccination (D0) in all subjects. Additional plasma was collected at D28 and D56 post-vaccination in children <5, day 28 (D28) in children 10–17 years old, D28 in LAIV3 vaccinated adults and at day 21 (D21) in IIV3 vaccinated adults.

2.3. Hemagglutinin specific IgG1, IgG2, IgG3 and IgG4 ELISA

An indirect ELISA was performed in order to determine the HA-specific IgG1, IgG2, IgG3 and IgG4 antibody concentrations in serum samples [17,18]. Ninety-six-well plates were coated with Influenza A/Texas/50/2012 (H3N2) -HA1 6xHis-tagged Hemagglutinin (1 μg/ml) (eEnzyme) or capture IgG antibody (0.3 μg/ml). Antibody concentrations were calculated using IgG1, IgG2, IgG3 and IgG4 standards and linear regression of the log-transformed readings.

2.4. Hemagglutinin specific IgA1 ELISA

ELISA plates were coated as previously described for the IgG1 detection except monoclonal goat anti-human IgA (Sigma) (1 μg/ml) and horseradish peroxidase-conjugated monoclonal mouse anti-human IgA1 Abs (SouthernBiotech) were used as detection antibodies.

2.5. IgG avidity ELISA

Serum samples were evaluated for avidity of HA-specific IgG antibodies as previously described [17]. ELISA plates were coated with Influenza A/Texas/50/2012-HA1 6xHis-tagged Hemagglutinin (1 μg/ml) (eEnzyme). Serum samples were standardized to a dilution that gave an Optical Density of 0.7 ± 0.3 in a direct ELISA and 1.5 M Sodium thiocyanate (NaSCN) was added 1 h after the serum, followed by 1 h of incubation. The percentage of antibodies remaining after treatment with 1.5 M NaSCN was calculated as: $(OD_{450}$ *treated serum*$/OD_{450}$ *untreated serum)* × *100%.*

2.6. Statistics analysis

Data analysis was performed using GraphPad Prism version 5. Kruskal-Wallis test was used for multiple comparisons between the four groups. Wilcoxon and Friedman tests were used to

Table 1
Study demographics.

	Children <5	Children 10–17yrs	LAIV3 vaccinated adults	IIV3 vaccinated adults
Number	15	14	15	15
M/F (% Male)	11/4 (73%)	3/11 (21%)	5/10 (33%)	2/13 (13%)
Age, mean (range)	3.8 (3–5)	14.2 (10–17)	34.6 (19–59)	44.9 (26–64)
Previous influenza vaccination	4 (27%)[a]	7 (50%)[a]	5 (33%)[a]	15 (100%)[b]

[a] Pandemic H1N1 vaccination in 2009.
[b] Prior seasonal influenza vaccination and pandemic H1N1 vaccination in 2009.

compare pre- and post-vaccination data within each group. Correlation between the serological assays was performed using Spearman rank test. A p-value <0.05 was considered statistically significant.

3. Results

3.1. LAIV3 induces age-related H3N2 HI antibody responses in children and adults

We analyzed the seroprotective HI antibodies to H3N2 in children vaccinated with LAIV3 and in adults vaccinated with LAIV3 or IIV3. Prior to vaccination, adults vaccinated with IIV3 had the highest HI titers (GMT 68) with 73% having pre-existing seroprotective titers (HI titers $\geqslant$ 40) compared to 40% (GMT 27.4), 43% (GMT 21.2) and 27% (GMT 12.6) in children <5 years old, children 10–17 years old and LAIV3-vaccinated adults, respectively (Fig. 2A). Vaccination resulted in a significant increase in HI titers in all groups except for LAIV3-vaccinated adults ($p < 0.05$) (Fig. 2A and B). High titers and seroprotection rates were observed after vaccination in 87% (GMT 220.5), 86% (GMT 147.3) and 87% (GMT 89) of children <5 years, children 10–17 years and IIV3 vaccinated adults, respectively. In contrast, the post-vaccination seroprotection rate in LAIV3-vaccinated adults increased slightly but was low at 40% (GMT 20.8). The HI titers induced by LAIV3 in adults were significantly lower than the other 3 groups ($p < 0.01$). Furthermore, the post-vaccination HI titers in the LAIV3-vaccinated subjects negatively correlated with age (Spearman's $r = -0.57$, $p < 0.0001$) (Fig. 2C).

3.2. LAIV3 after two doses in young children and IIV3 in adults induced comparable hemagglutinin-specific IgG1 responses

Since the HI assay does not differentiate between the IgG subclasses, we quantified the H3N2 HA1-specific IgG1, IgG2, IgG3 and IgG4 antibodies in an ELISA assay. Pre-vaccination, HA1-specific IgG1 levels were comparable between the children and adults (Fig. 3A). In children <5 years old, a second vaccine dose was required to induce a significant increase in IgG1 levels. In children 10–17 years old one dose of LAIV3 was sufficient to induce a significant increase in HA1-specific IgG1 concentrations. However in adults, no change in HA1-specific IgG1 concentrations was observed after LAIV3 vaccination. IIV3 induced a significant increase in HA1-specific IgG1 in adults (Fig. 3A) and hence significantly higher fold increase in IgG1 (mean 3.43 fold) than LAIV3 in adults (mean 0.90 fold) ($p = 0.008$) (Fig. 3B). IIV3 induced significantly higher IgG1 than one dose of LAIV3 in both older children (10–17 years) and adults ($p < 0.05$) (Fig. 3A).

Overall, IgG3 was detected at very low concentrations in a few subjects but was not detectable in most vaccinees (Supplementary Fig. 1). HA1-specific IgG2 and IgG4 subclasses were absent in all subjects tested (data not shown).

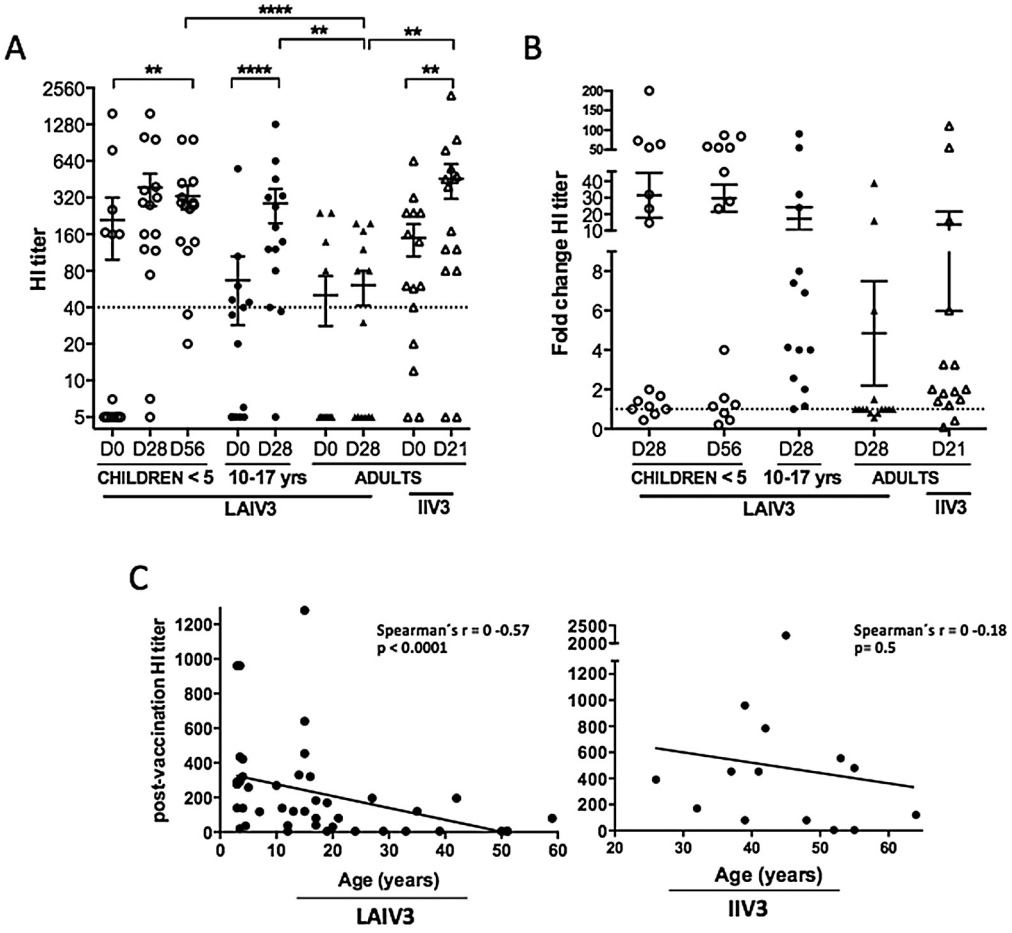

Fig. 2. Hemagglutination inhibition response. (A) Geometric mean HI titers against influenza A H3N2/Texas virus pre- and post-vaccination in children <5 (open circles), children 10–17 years old (closed circles), LAIV3 vaccinated adults (closed triangles) and IIV3 vaccinated adults (open triangle). The dotted line indicates a titer of 40, which is considered protective. Each symbol represents the GMT of all the titers at each time-point ± SEM (bars). (B) Pre- to post-vaccination fold change in HI titers. (C) Correlation between age and post-vaccination HI titers in LAIV3 and IIV3 vaccinated subjects. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$, $^{****}p < 0.0001$.

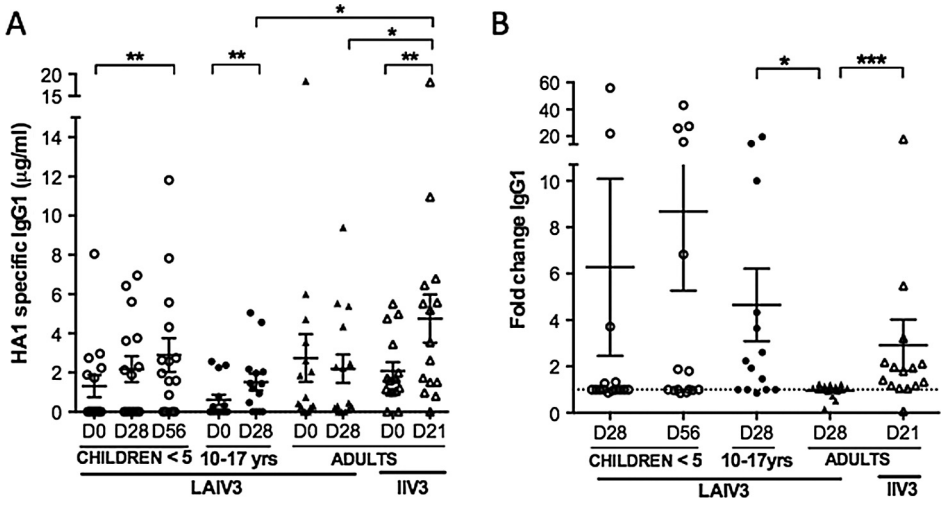

Fig. 3. HA1-specific IgG1 response. (A) The concentration of HA1-specific IgG1 was measured in pre- and post-vaccination plasma of children <5 (open circles), children 10–17 years old (closed circles), LAIV3 vaccinated adults (closed triangles) and IIV3 vaccinated adults (open triangle). Each symbol represents an individual. (B) Fold induction of post-vaccination IgG1 concentrations over pre-vaccination IgG1 concentrations. ∗ indicates statistically significant differences in responses $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Since most antibodies generated after vaccination are IgG and the HI assay detects HA specific antibodies, we analyzed the relationship between the HA1-specific IgG1 and HI response. We found a significant correlation between HI titers and HA1-specific IgG1 in LAIV3-vaccinated children and adults post-vaccination (Spearman's $r > 0.5$, $p < 0.01$). However, we observed no relationship between the HI titers and HA1-specific IgG1 response in IIV3-vaccinated adults (Supplementary Fig. 2).

3.3. High HA-specific IgA1 responses induced by vaccination in children but not in adults

We investigated whether LAIV3 or IIV3 induced HA1-specific IgA1 antibodies in children and adults. Pre-vaccination, HA1-specific IgA1 was detectable in all the groups. Significantly higher HA1-specific IgA1 levels were found in children <5 years old compared to older children aged 10–17 years ($p < 0.05$) (Fig. 4A). Vaccination resulted in a significant increase in HA1-specific IgA1 concentrations in children but not in adults (Fig. 4A). Young children who received two vaccine doses had significantly higher HA1-specific IgA1 after the second dose than older children and adults who all received one vaccine dose ($p < 0.05$). Although LAIV3 or IIV3 did not result in a significant increase in IgA1 levels in adults, the IgA1 concentrations and fold changes were maintained at similar levels to those in older children (10–17 years) who also received one vaccine dose (Fig. 4B).

3.4. Vaccination induced high avidity antibodies in children and adults

To assess the difference in quality of the IgG antibodies induced by LAIV3 and IIV3, the avidity of HA1-specific IgG antibodies was measured. The percentage of bound IgG antibodies remaining after treatment with 1.5 M NaSCN was calculated. The avidity of HA1-specific IgG antibodies was comparable between young and older children, as well as between the two adult groups, pre-vaccination (Fig. 5A). However, the avidity of HA1-specific IgG antibodies in older children (10–17 years) was significantly lower than that of the two adult groups ($p < 0.05$). Vaccination resulted in a significant increase in antibody avidity in children <5 years old, children 10–17 years old and IIV3-vaccinated adults but not

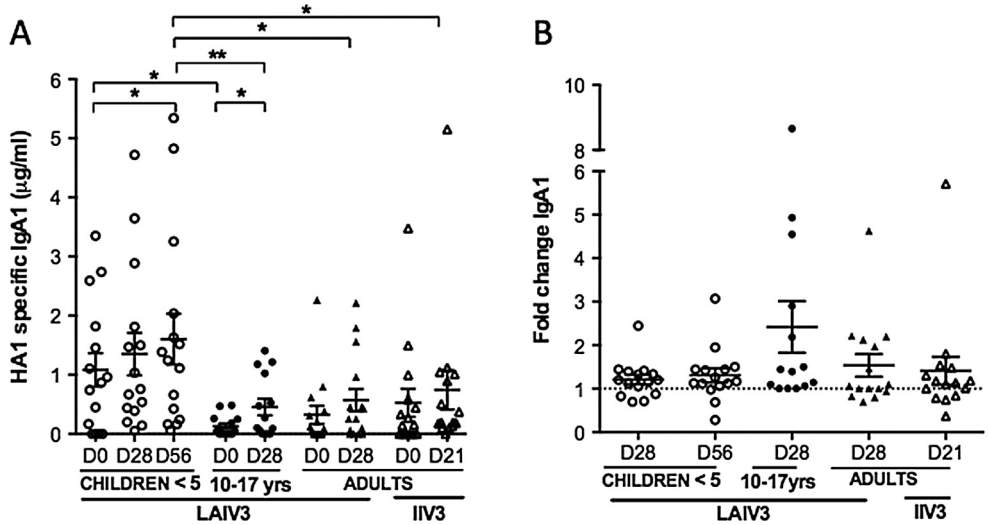

Fig. 4. HA1-specific IgA1 response. (A) IgA1 antibodies specific to HA1 in children <5 (open circles), children 10–17 years old (closed circles), LAIV3 vaccinated adults (closed triangles) and IIV3 vaccinated adults (open triangle) pre- and post-vaccination. (B) Pre- to post-vaccination fold change in HA1 specific IgA1. $^{*}p < 0.05$; $^{**}p < 0.01$.

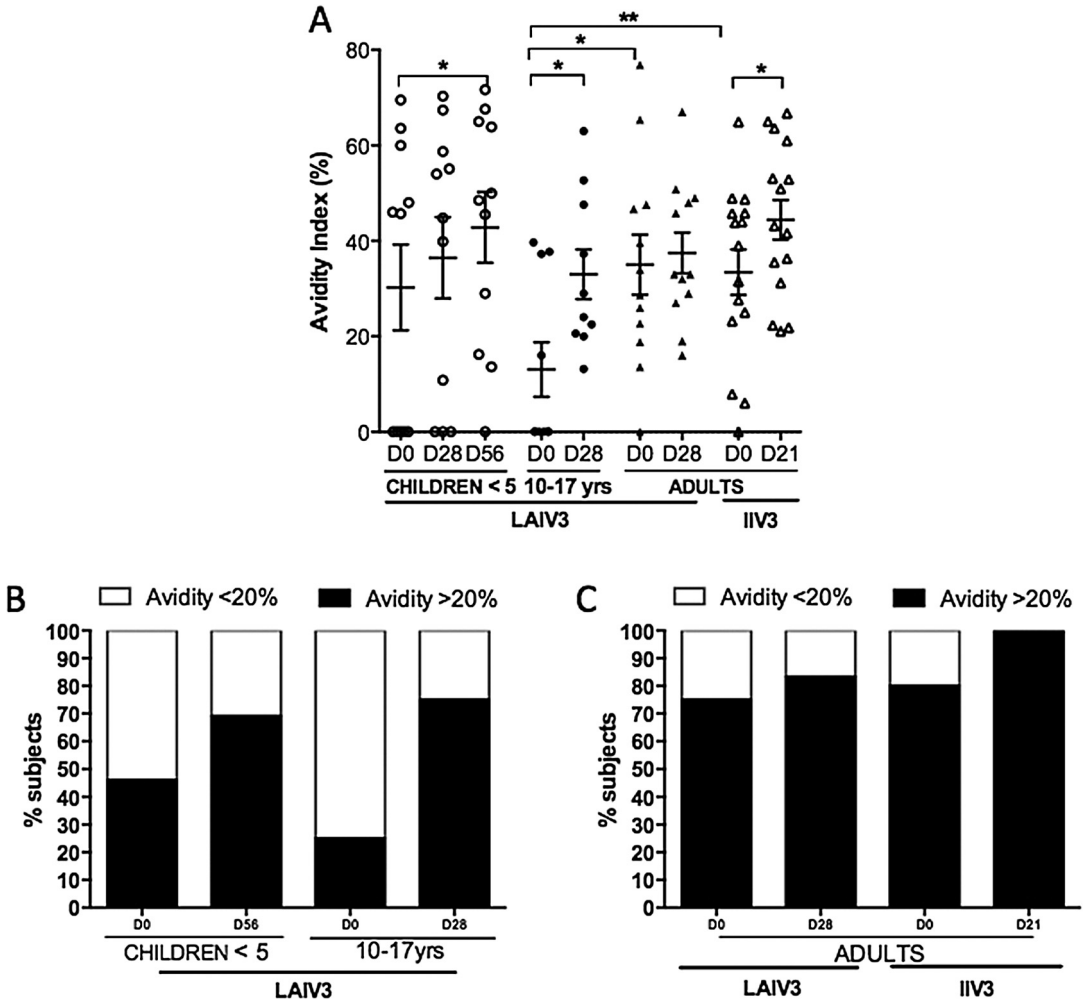

Fig. 5. Hemagglutinin specific IgG antibody avidity. (A) The avidity of HA1 specific total IgG antibodies was measured in an avidity ELISA assay for children <5 (open circles), children 10–17 years old (closed circles), LAIV3 vaccinated adults (closed triangles) and IIV3 vaccinated adults (open triangle) pre- and post-vaccination. Standardized sera were treated with 1.5 M NaSCN and the percentage of HA1-specific IgG antibodies remaining bound after 1.5 M NaSCN treatment was measured as (absorbance of treated samples ÷ absorbance of untreated samples) × 100%. The line represents the mean avidity index at each time-point ± SEM. The stacked column graphs represent the percentage of (B) children and (C) adults with high avidity antibodies (avidity index ⩾ 20%). The percentage of subjects with antibodies with <20% avidity are shown in white while those with avidity index ⩾ 20% are shown in black. $^{*}p < 0.05$; $^{**}p < 0.01$.

in LAIV3-vaccinated adults, with avidity indices of 46.8%, 33%, 37.5% and 44.4%, respectively (Fig. 5A). Even though the avidity of IgG antibodies in LAIV3-vaccinated adults did not increase, antibodies post-vaccination were characterized by high avidity, comparable to the other three groups.

We used a cut-off avidity index of 20% to determine which subjects had high avidity antibodies. Pre-vaccination, a higher number of adults compared to children showed high avidity (>20% avidity index) antibodies. The frequency of children <5 years old, children 10–17 years old, LAIV3-vaccinated adults and IIV3-vaccinated adults with pre-existing high avidity antibodies were 46%, 25%, 75% and 80%, respectively (Fig. 5B and C). Post-vaccination, the proportion of children with high avidity IgG increased from 46% to 69% and from 25% to 75% in <5 year olds and 10–17 year olds, respectively. Both LAIV3-vaccinated and IIV3-vaccinated adults maintained elevated antibody avidity with 83% and 100% having high avidity antibodies, respectively (Fig. 5C).

Interestingly, we found a positive correlation between the avidity of pre-existing HA-specific IgG and post vaccination HA1-specific IgG1 response in children <5 (spearman's $r = 0.71$, $p = 0.0068$) and children 10–17 years old (spearman's $r = 0.72$, $p = 0.01$) (Supplementary Fig. 3). However, this correlation was not observed in either LAIV3 or IIV3 vaccinated adults.

4. Discussion

Annual influenza vaccination is the most effective method to prevent infection especially in subjects prone to develop secondary complications, such as young children, pregnant women, people with chronic medical conditions and the elderly [18]. Both mucosal and systemic antibodies have been previously shown to be involved in the protection against influenza infection [11,19,20]. Antibodies specific to the HA1 domain of HA, which contains the receptor-binding site, are important for viral neutralization [21,22]. However, the immune response after vaccination may be influenced by several factors, including vaccine type and age of recipients [5,23,24]. Here, we evaluated the quantity as well as the quality of the antibodies specific to H3N2 HA induced by LAIV3 and IIV3 in 4 different participant groups, varying by age and vaccine type.

LAIV vaccines contain live, cold-adapted influenza viruses that only replicate in the mucosal membranes of the upper respiratory tract (<33 °C), causing only mild subclinical infection in humans

[26,27]. LAIV promotes a robust antibody response in young children, especially in individuals that are seronegative pre-vaccination [28–30]. Previous studies demonstrated that LAIV3 was more effective than IIV3 in children aged from 6 months to 17 years old [25,26]. A study comparing the efficacy and safety of LAIV3 *versus* IIV3 in children with recurrent respiratory tract infections showed that LAIV3 resulted in a 53% reduction in influenza cases by antigenically matched vaccine strains compared to IIV3 [25]. Another study by Fleming et al. reported 35% fewer influenza cases in LAIV3 recipients compared to IIV3 recipients [26]. Although at present no immunological correlates of protection are available for LAIV, our HI assay results confirm that LAIV3 induces a significant antibody response in children of all ages.

A study by Treanor et al. showed that IIV3 induced higher HI titers than LAIV3 in adults, with titers induced by LAIV3 being comparable to placebo. However, after challenge with wild type virus of vaccinated adults with HI titers $\leqslant 8$, laboratory confirmed illness occurred in 13%, 7% and 45% of IIV3, LAIV3 and placebo vaccinated adults, respectively [27]. They demonstrated that despite low HI titers, LAIV3 had comparable efficacy to IIV3 in adults. We showed that IIV3 induces significantly higher HI titers than LAIV3 in adults, with LAIV3 resulting in no increase in titers. Despite no increase in HI titers after LAIV3 vaccination in adults, they could still be protected by other immune mechanisms [28–30].

In Norway, seasonal influenza vaccination is only recommended for children with high-risk conditions and the children in our study were healthy and therefore not previously vaccinated. Only 27% of children <5 years old and 50% of children 10–17 years old had received pandemic H1N1 influenza vaccination in 2009. However, six children <5 years had high HI titers (>160) and IgG1 levels against H3N2 virus. Also five children, 10–17 years old had HI titers > 40. These children are likely to have been previously exposed to H3N2 through natural infection explaining the high HI titers.

In this study, we investigated the IgG subclass response to H3N2 HA, since it is the most dominant serum immunoglobulin class induced after vaccination [25]. IgG1, along with IgG3, are involved in critical immunologic functions such as complement fixation, opsonization, antibody-dependent cellular cytotoxicity and virus neutralization [34]. In general, we detected elevated IgG1 levels in our subjects, whereas IgG3 levels were low post-vaccination. Both unvaccinated and previously vaccinated children and adults had pre-existing HA1-specific IgG1 reflecting previous exposure to antigenically similar influenza viruses [31,32]. Based on the significant increase in IgG1 antibodies in children, LAIV3 appears to be efficient in both priming the H3N2 response in the children with no pre-existing titers and boosting this response in children with pre-existing titers.

IIV3 vaccine elicits a more robust increase in serum IgG antibodies, with only a minor induction of local IgA response [33,34]. Conversely, LAIV induces higher local IgA response at the nasal epithelium and lower systemic antibody responses [38]. Since the LAIV mimics a natural infection, which stimulates a local IgA response providing protection at the local mucosa where the influenza virus starts its infection cycle. Children who had high HI titers also had high IgA1 levels and the higher pre-vaccination IgA1 observed in children <5 compared to older children could be due to the difference in numbers of children who had previous infection with H3N2 (supplementary Fig. 4). Our study of systemic IgA1 response revealed that whereas no change was observed after LAIV3 or IIV3 vaccination in adults, a significant increase was found in serum IgA1 in children after LAIV3. Particularly, younger children receiving two vaccine doses of LAIV3 showed significantly higher IgA levels compared to older children (10–17 years) who received a single dose of vaccine. The IgA1 response detected in our study could also be due to spill over of IgA from the site of vaccination at the local mucosa. However, we did not measure the nasal antibody response. A study by Boyce et al. demonstrated a positive correlation between nasal and serum IgG and IgA responses in adults intranasally vaccinated with inactivated vaccine [35]. Another study demonstrated elevated serum antibody titers positively correlated with nasal antibody levels. In addition, LAIV increased mucosal IgA but not systemic IgG in adults [36]. Our results are in agreement with these observations, as LAIV3 did not induce a significant increase in systemic IgG1 or IgA1 in adults. An earlier investigation showed that pre-existing nasal IgA, detected almost exclusively in subjects naturally infected or vaccinated with LAIV, was associated with protection [37]. Adults would have had a number of exposures to influenza in their lifetime either through vaccination or infection. It is plausible that pre-existing antibodies were present in the nasopharyngeal mucosa of the adults before vaccination, which may limit both intranasal infection and replication of LAIV, resulting in a lower antibody response as observed in our LAIV3-vaccinated adults [38].

The pre-existing HA1-specific IgG antibodies in children 10–17 years old had low avidity compared to adults. The high antibody avidity observed pre-vaccination in a few children and most adults may be due to pre-existing memory generated by previous infection. Avidity could indicate the priming of immunological memory as vaccination results in antibody maturation and hence generation of antibodies with increased avidity as we detected in the present study [37,39]. Priming and subsequent boosting of the antibody response results in a gradual increase in high affinity antibodies. Of note is that adults who received the IIV3 were healthcare workers who are offered yearly influenza vaccination and are also likely to come in contact with infected patients. We have previously reported that repeated annual vaccination in healthcare workers persistently boosted the avidity of influenza-specific IgG antibodies [40]. The high avidity antibodies were maintained in LAIV3-vaccinated adults, although they did not increase in quantity or quality post-vaccination. Interestingly, the avidity of pre-existing antibodies predicted the IgG1 response in children. This suggests that vaccination and infection outcome in children likely correlates with the quality of the antibodies and their ability to restrict virus replication to the upper respiratory tract. In adults, the elevated pre-existing IgG avidity was not associated with a higher IgG1 concentration after vaccination, suggesting that a low number of high avidity memory B cells could be sufficient for the maintenance of protection in previously vaccinated or exposed individuals.

This study was limited by several factors. First, small number of subjects was included per group, thus limiting the statistical comparisons that could be made. Another limitation is that we did not measure mucosal IgA; which is particularly important after LAIV however, our study objective was to measure both serum IgG and IgA antibody responses.

In conclusion, our findings confirm that LAIV3 promotes a stronger systemic antibody response in children than in adults. In adults, IIV3 induces better antibody responses compared to LAIV3, but comparable antibody response to that induced in LAIV3-vaccinated children. The different mechanisms of action of LAIV3 *versus* IIV3, may explain the relative efficacy between the two vaccines in children and adults. In children, the avidity of pre-existing serum antibodies likely plays a role in determining the antibody response to infection. Our results suggest that exposure history and the type of vaccine play a significant role in determining the antibody response.

Contributors

A.M. performed the experiments and was involved in interpreting the data. S.M.T. was involved in the daily supervision of the study, interpreting the data and prepared the manuscript. K.G.I.

M. was involved in sample collection and interpretation of the results. A.J.-L. and E.M. were involved in interpretation of the results. R.J.C., K.A.B. and E.M. contributed to the study design, protocol design and interpretation of the results. All authors critically reviewed the manuscript and approved the final article.

Conflict of interest

None.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.10.024.

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