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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.

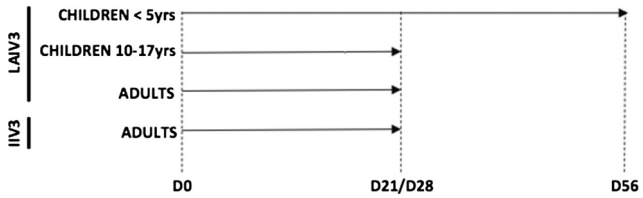

Fig. 1. Study design. Fifteen healthy children <5 years old, 14 children aged 10–17 and 15 adults were vaccinated with Live Attenuated Influenza Vaccine (LAIV3). In addition, 15 adults were vaccinated with Trivalent Inactivated Influenza Vaccine (IIV3) as a control. Children <5 received 2 vaccine doses, 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.