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Influenza A haemagglutinin specific IgG responses in children and adults after seasonal trivalent live attenuated influenza vaccination

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ABSTRACT

Influenza is a major respiratory pathogen and vaccination is the main method of prophylaxis. In 2012, the trivalent live attenuated influenza vaccine (LAIV3) was licensed in Europe for use in children. Vaccine-induced antibodies directed against the main viral surface glycoprotein, haemagglutinin (HA), play an important role in virus neutralization through different mechanism. The objective of this study was to dissect the HA specific antibody responses induced after LAIV3 immunization to the influenza A viruses in children and adults.

Plasma was collected from 20 children and 20 adults pre- and post-LAIV3 vaccination (up to a year) and analysed by the haemagglutination inhibition (HI) and ELISA assays. We found that LAIV3 boosted the HA specific IgG response against the head and the full-length of H3N2 in children, but not adults. Adults had higher levels of pre-existing stalk antibodies (towards H3N2 and H1N1), but these were not boosted by LAIV3. Importantly, we observed a trend in boosting of H1N1 HA stalk specific antibodies in children after LAIV3. Whereas, heterosubtypic H5 and H7 full-length HA specific antibodies were not boosted in either children or adults. In conclusion, LAIV3 elicited H3-head and low levels of H1 stalk specific antibody responses in children, supporting the prophylactic use of LAIV in children.

1. Introduction

Influenza viruses cause annual seasonal outbreaks or epidemics, with occasional pandemics occurring at unpredictable intervals. Influenza infects all age groups, although the burden of hospitalization is highest in very young children and the elderly [1–3]. Vaccination is the main method of influenza prophylaxis with either inactivated influenza vaccine (IIV) or live attenuated influenza vaccine (LAIV). Although used in Russia for decades, the LAIV was first licensed in the USA in 2003 for children and adults (2–49 years old), and in 2012 in Europe for children (2–17 years old). LAIV is administered as a nasal spray and replicates in the upper respiratory tract, mimicking natural infection and inducing both humoral and cellular immune responses [4]. Trivalent LAIV (LAIV3) has been reported to have a higher efficacy in young children than intramuscular IIV and thus provide greater protection against influenza-associated severe complications [5–8]. Importantly, the immune response after seasonal IIV is strain-specific, whereas LAIV3 provides better protection against mismatched strains [8,9].

The haemagglutinin is the major viral surface antigen, consisting of two domains; the globular head and the stalk domains, with a disulphide bride between C52 and C277 (H3 numbering) being the demarcation line between the two domains [10]. The immuno-dominant globular head contains the receptor-binding site and the antigenic sites, which undergo continuous antigenic drift. The membrane proximal HA stalk is highly conserved and contains a conformation-dependent fusion-peptide [11]. Antibodies directed against the stalk are broadly neutralizing, recognizing divergent and heterosubtypic strains from either group 1 (including H1 and H5) or group 2 (including H3 and H7) viruses [12–14] and provide *in vivo* protection from viral challenge in animal models [14,15]. Antibodies can be boosted to the more conserved but less immunogenic stalk, when a virus has a highly divergent HA

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head from a previously circulating strain, such as the 2009 pandemic or avian H5N1 virus [13,14,16]. Therefore, the conserved HA stalk is a promising target for development of a future universal influenza vaccine.

Conventional IIVs predominantly induce strain-specific antibody to the HA head, but the type of HA response LAIV3 elicits is yet to be fully defined. In this study, we dissected the HA head and stalk-specific antibody responses to the homologous vaccine (H1N1 and H3N2) and heterologous avian (H5N1 and H7N9) viruses after intranasal seasonal LAIV3 vaccination in children and adults.

2. Material and methods

2.1. Participants and study design

Twenty children (3–17 years old) and 20 adults (21–59 years old) were intranasally vaccinated (0.1 mL per nostril) with seasonal LAIV3 (Fluenz, Astra Zeneca, Liverpool, UK). Children received one (≥9 years old, n=6) or two doses (<9 years old, n=14) of LAIV3 in 2012 at a four-week interval [4]. Adults received one dose of LAIV3 in 2013. The study had ethical and regulatory approval (EUDRACT2012-002848-24, www.clinicaltrials.gov; NCT01866540) and the exclusion criteria are published [4]. Plasma was collected at 0 (pre-vaccination), 28, 56, 180 and 360 days post-vaccination, aliquoted and stored at −80 °C.

2.2. Vaccine

LAIV3 (Fluenz) contained 10^7 fluorescent focus units (FFU) of A/California/7/2009(H1N1)pdm09-like and A/Victoria/361/2011 (H3N2)-like strains in both seasons, with either B/Wisconsin/1/2010-like or B/Massachusetts/2/2012-like in the 2012 or 2013 seasons, respectively.

2.3. Recombinant haemagglutinin proteins

The influenza A full-length and chimeric haemagglutinin proteins were prepared by using the baculovirus expression system (Table 1) [16]. The cH6/1 contained the globular head domain from A/mallard/Sweden/81/02 (H6N1) and the stalk domain from A/PuertoRico/8/34 (H1N1). The cH4/3 contained the H4 globular head domain from A/duck/Czech/1956 (H4N6) in combination with the H3 stalk domain from A/Perth/16/09 (H3N2). The HA1 proteins were purchased from eEnzyme, USA and were used as proxy for the head domain.

Table 1
Source of virus antigens.

	Strain	Haemagglutinin (HA)
Group 1	A/California/04/09 (H1N1)	Full-length H1
		H1 HA1 (head proxy)
	Chimeric protein: stalk of A/PuertoRico/8/34 (H1N1), globular head from A/mallard/Sweden/81/02 (H6N1)	Stalk (cH6/1)
	A/Vietnam/1203/04 (H5N1)	Full-length H5
Group 2	A/Victoria/361/11 (H3N2)	Full-length H3
	A/Texas/50/2012 (H3N2)	H3 HA1 (head proxy)
	Chimeric protein: stalk of A/Perth/16/09 (H3N2), globular head from A/duck/Czech/1956 (H4N6)	Stalk (cH4/3)
	A/Shanghai/1/13 (H7N9)	Full-length H7

2.4. Haemagglutination inhibition (HI) assay

The HI assay was conducted using serial 2-fold dilutions of receptor destroying enzyme (RDE, Seiken, Japan) treated plasma (starting dilution 1:10) and 0.7% turkey blood cells, as previously described [4].

2.5. Enzyme-linked immunosorbent assay (ELISA)

The ELISA was conducted using the full-length, head or chimeric HAs as previously described [17]. The end-point titres were calculated using the mean of the blank plus three standard deviations as a cut off [13].

3. Statistics

Statistical differences after vaccination were analysed using the linear mixed model in STATA/IC 14.1 for Mac (StataCorp, USA). The Wilcoxon test was used for the head/stalk distribution after vaccination and the nonparametric Mann-Whitney test for comparing children and adult responses in HI (GraphPad Prism® V6f for Mac, GraphPad Software, USA). The children and adult sampling points were also compared using paired student's *t*-test and *t*-test with Welch correction. $P < 0.05$ was considered significant.

4. Results

Twenty children (median 4.5 years old) and twenty adults (median 33.5 years old) were immunized with seasonal LAIV3 and sequential blood samples were collected up to one year post-vaccination (Fig. 1). Fourteen children (70%) (median age 3.8 years old) received two doses of vaccine, whilst the remaining 6 children (30%) (median age 15.5 years old) and adults received only one dose (Table 2). Ten adults (50%) and nine children (45%) were vaccinated in 2009 with the AS03-adjuvant pandemic H1N1 vaccine (Table 2). One child's (5%) mother was also vaccinated while pregnant in 2009, and only one child (5%) had previously been vaccinated with trivalent IIV.

4.1. LAIV3 boosts H3N2 haemagglutination inhibition antibody responses in children

We examined the plasma HI antibody response against the influenza A H1N1 and H3N2 vaccine strains after LAIV3. An HI titre of ≥40 is considered to protect 50% of individuals from disease. Pre-vaccination, fifteen children (75%) had protective antibody titres against H1N1 (Fig.2A), which were not boosted after vaccination. Five children (25%) remained below the protective HI threshold after the first vaccination, of whom one responded after the second dose (HI titre 69) and the remaining 4 children (20%) did not respond (HI < 10) vaccination. Children had significantly higher HI titres than adults pre- to 180 days post-vaccination. Pre-vaccination, ten adults had HI titres below the protective threshold of whom nine were seronegative (HI < 10). Vaccination did not generally boost antibody titres in adults, except two adults who responded with protective HI titres. No change in fold change in HI titres was observed to H1N1 after LAIV3 (Fig. 2C).

For the H3N2 strain, 10 (50%) of the 20 children had a pre-vaccination HI titre of <40, of whom 8 (40%) were seronegative (HI titre < 10) (Fig. 2B). After the first LAIV3 dose, there was a significant increase ($P < 0.0001$) in HI titres, which remained significantly elevated above pre-vaccination levels after the second dose (day 56) ($P < 0.0001$) and until day 360 ($P < 0.01$). Thirteen (65%) adults had pre-vaccination antibodies below the protective titre (HI<40) to H3N2. LAIV3 elicited protective HI titres in two

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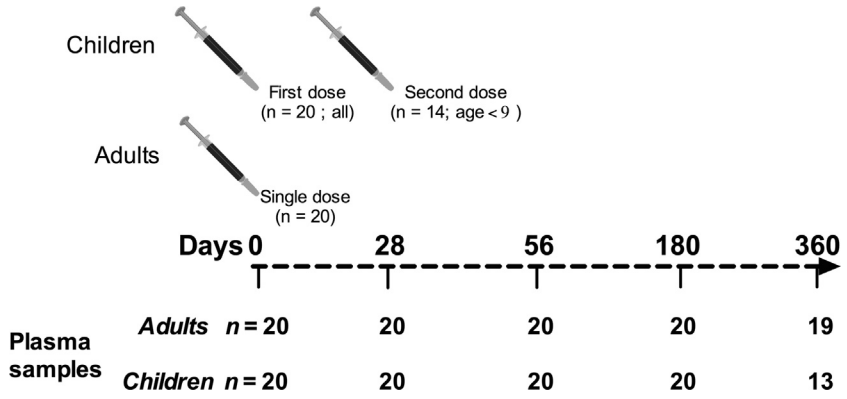

Fig. 1. The study design. Children were vaccinated with 1 (n = 20) or 2 (n = 14 children, age < 9 years old) doses of LAIV, whilst adults received one dose of LAIV. Plasma samples were collected pre (0) and at 28, 56, 180 and 360 days post vaccination. The number (n) of children and adults providing samples at each time point is shown.

Table 2
Baseline demographics of the patient cohort.

	Children	Adults
Number of participants (n)	20	20
Median age by year (Range)	4.5 (3–17)	33.5 (21–59)
Male/Female (% Female)	9/11 (55%)	6/14 (70%)
Single dose (%)	6 (30%)	19 (95%)
Two doses (%)	14 (70%)	1 (5%)
Pandemic vaccination in 2009 (n (%))	9 (45%)	10 (50%)

adults (10%) post-vaccination, only one of which was maintained above the protective titre up to 1 year. Children had significantly higher fold changes after LAIV3 than adults, and these were maintained in children up to day 360 whereas low increases were observed up to day 56 in adults (Fig. 2D). In summary, LAIV3 significantly boosted the HI response to H3N2 in children. Generally, no H1N1-specific HI response was boosted in either children or adults.

4.2. IgG responses to full-length H3N2 were boosted in children after LAIV3

The IgG response was measured by ELISA to the homologous (H1 and H3) and heterologous (H5 and H7) full-length influenza A HAs (Table 1). High pre-vaccination IgG titres to the full-length H1 HA were detected in both the children and adults, and LAIV3 vaccination did not boost these antibodies (Fig.3A). No correlation was observed between pre-vaccination IgG and fold-increase 28 days post-vaccination in children against H1N1 (data not shown).

H3-specific antibodies were detectable pre-vaccination in all adults and in the majority of children to the full-length HA, although 5 children had low levels of antibodies (Fig. 3B). In the children, antibody titres increased significantly ($p < 0.0005$) at day 28 after LAIV3 immunization and were generally maintained up to day 360. Children's IgG titres were significantly higher than adults after one dose and up to one year post-vaccination. No changes in H3-specific antibody responses were observed in the adults after vaccination. Pre-existing IgG antibodies specific for H3 full-length HA significantly but inversely correlated with fold induction in children both at day 28 ($r = -0.8412$, $p < 0.0001$) and day 56 ($r = -0.8618$, $p < 0.0001$).

We further evaluated the heterosubtypic antibody response. H1 and H5 are both group 1 HAs and the H3 and H7 are group 2 HAs, with a similar conserved stalk domain. We observed a trend of higher pre- and up to 180 days post-vaccination antibody titres against the full-length H5 HA (Fig. 3C) in adults compared to children. The H5 antibody titres at day 360 were significantly different ($P < 0.05$) from earlier time points in both adults and children. No change in H3 stalk antibodies was observed after LAIV3 (Fig. 3D). However, adults had significantly higher H7 HA-specific antibody titres pre- and up to 180 days post-vaccination compared to children.

4.3. LAIV3 boosts H3 HA head-specific responses in children

We measured the IgG antibodies to the homologous influenza A vaccine HA heads and stalks using chimeric HAs. The H1 head-specific antibody titres were significantly higher in children than the adults (Fig. 4A) pre-vaccination, although the LAIV3 did not boost these antibodies. Adults only had a slight increase in head antibody but this decreased by day 360. Pre-vaccination, H3 head specific antibodies were comparable between children and adults (Fig. 4B). In children, H3 head specific antibodies increased significantly ($p < 0.05$) after one dose (day 28) and were maintained up to one year post-vaccination (Fig. 4B). However, no boost in H3 head antibodies was observed after vaccination in adults. Children had significantly higher head H3 specific antibodies after LAIV3 immunization than adults.

The stalk-specific antibody response was assessed using chimeric group 1 (cH6/1) and group 2 (cH4/3)) HAs containing exotic head domains derived from avian viruses, which do not cause human infection. For the H1 stalk, adults had significantly higher stalk antibody titres compared to children pre-vaccination and up to day 180 ($p < 0.05$). There was a trend of an increase in the H1 stalk antibody response in children at days 28 and 56 post-vaccination, but not in adults (Fig. 4C). Adults had significantly higher H3 stalk-specific antibodies pre-vaccination than the children. LAIV3 immunization did not boost H3 stalk-specific IgG in children or adults. H3 stalk-specific antibody titres decreased significantly ($P < 0.05$) in adults at days 180 and 360 compared to pre-vaccination titres (Fig. 4D). In summary, circulating H3N2 head-specific IgG responses were boosted and low levels of H1N1 stalk-specific antibody responses were induced in children, but not adults.

4.4. Comparative distribution of head- and stalk-specific IgG after LAIV3

The HA specific IgG distribution was compared by calculating the ratio of the antibodies to the head domain and the stalk domain in children and adults. Children, who had previously been infected or vaccinated with the H1N109pdm virus, but with limited exposure history to other influenza H1s, had more H1 head-specific

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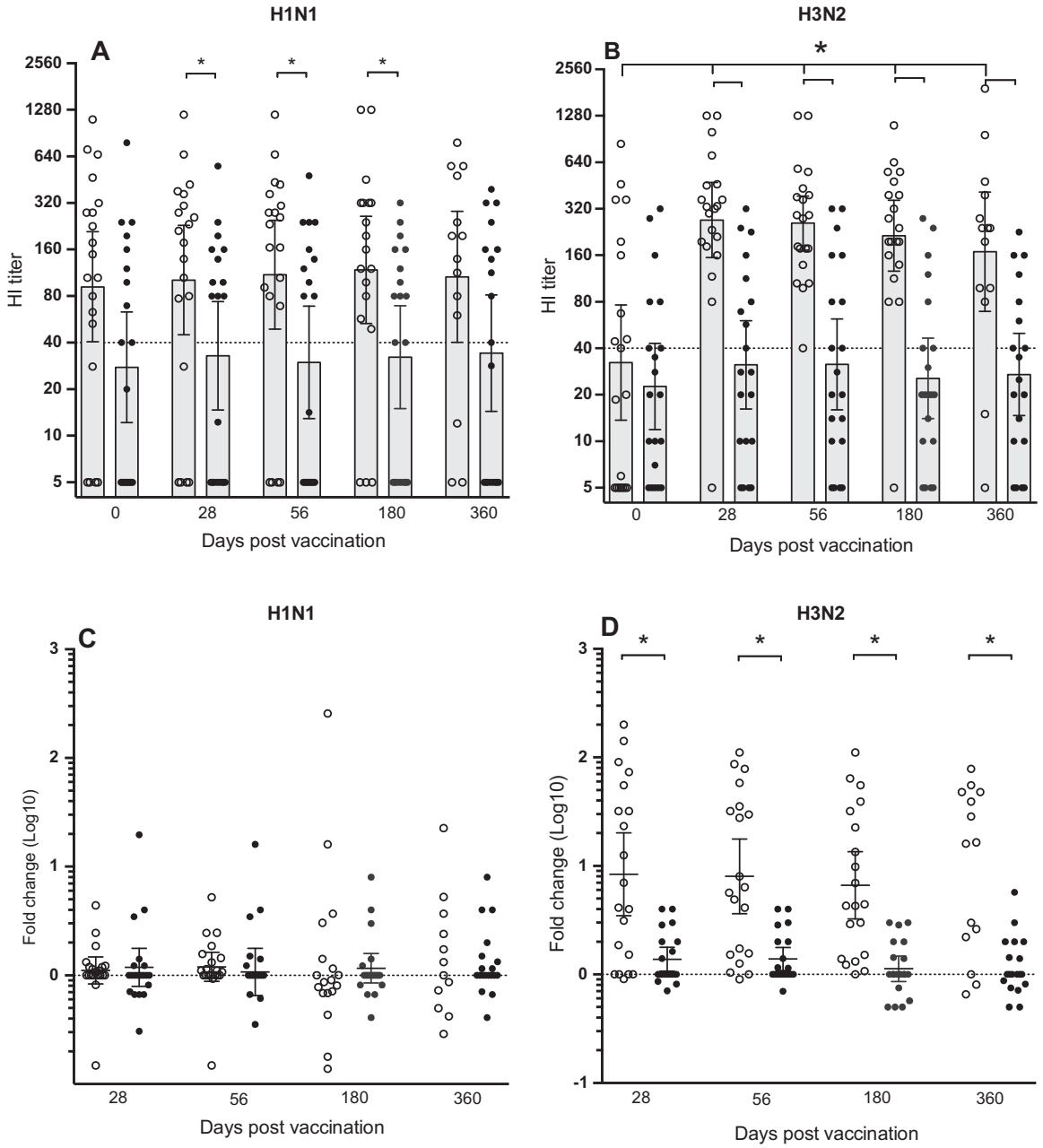

Fig. 2. The haemagglutination inhibition (HI) antibody responses in children and adults after live attenuated influenza vaccination. HI titres were measured towards the homologous influenza A/California/7/09 (H1N1) (A) and A/Victoria//361/11 (H3N2) (B) vaccine strains in both children and adults after live attenuated influenza vaccination. The dotted line shows an HI titre of 40, considered the protective level. The fold increase in children from pre-vaccination antibody titres to H1N1 (C), and H3N2 (D) homologous viruses. Ratios above or below 1 indicate higher or lower post-vaccination HI titres compared to pre-vaccination titres, respectively. Blood was collected at 0 (pre), 28 (after 1st dose), 56 (after 2nd dose in children < 10 years old), 180 and 360 days post vaccination. Each circle represents the HI response of one individual with the bar showing the group geometric mean HI titres ± 95% confidence interval. Open circles are children while filled circles are adults.. The nonparametric Mann-Whitney test was used to investigate statistical significance between children and adults. The linear mixed model was also used to investigate the change from pre-vaccination antibody titres up to one year for both children and adults; $^{*}p < 0.05$.

antibodies pre-vaccination, which decreased post-vaccination. However, in adults who had previously experienced several natural H1N1 infections and/or vaccination, HA stalk-specific antibody dominance was observed (Fig. 4E). The children had significantly higher ratio throughout the study than adults. Both children and adults had a H3 head dominant response (Fig. 4F). An increase in H3 head specific-antibodies after LAIV3 immunization was observed in children, leading to a significantly higher ratio in children than adults post-vaccination.

5. Discussion

The licensure of LAIV in Europe in 2012 expanded available prophylaxis for influenza, offering an attractive easily administered nasal spray vaccine for children. In Norway, annual seasonal influenza vaccination is only recommended for high-risk populations and thus most of our volunteers had not previously received seasonal influenza vaccination. In this study, we dissected the influenza A HA-specific antibody response after the newly licensed

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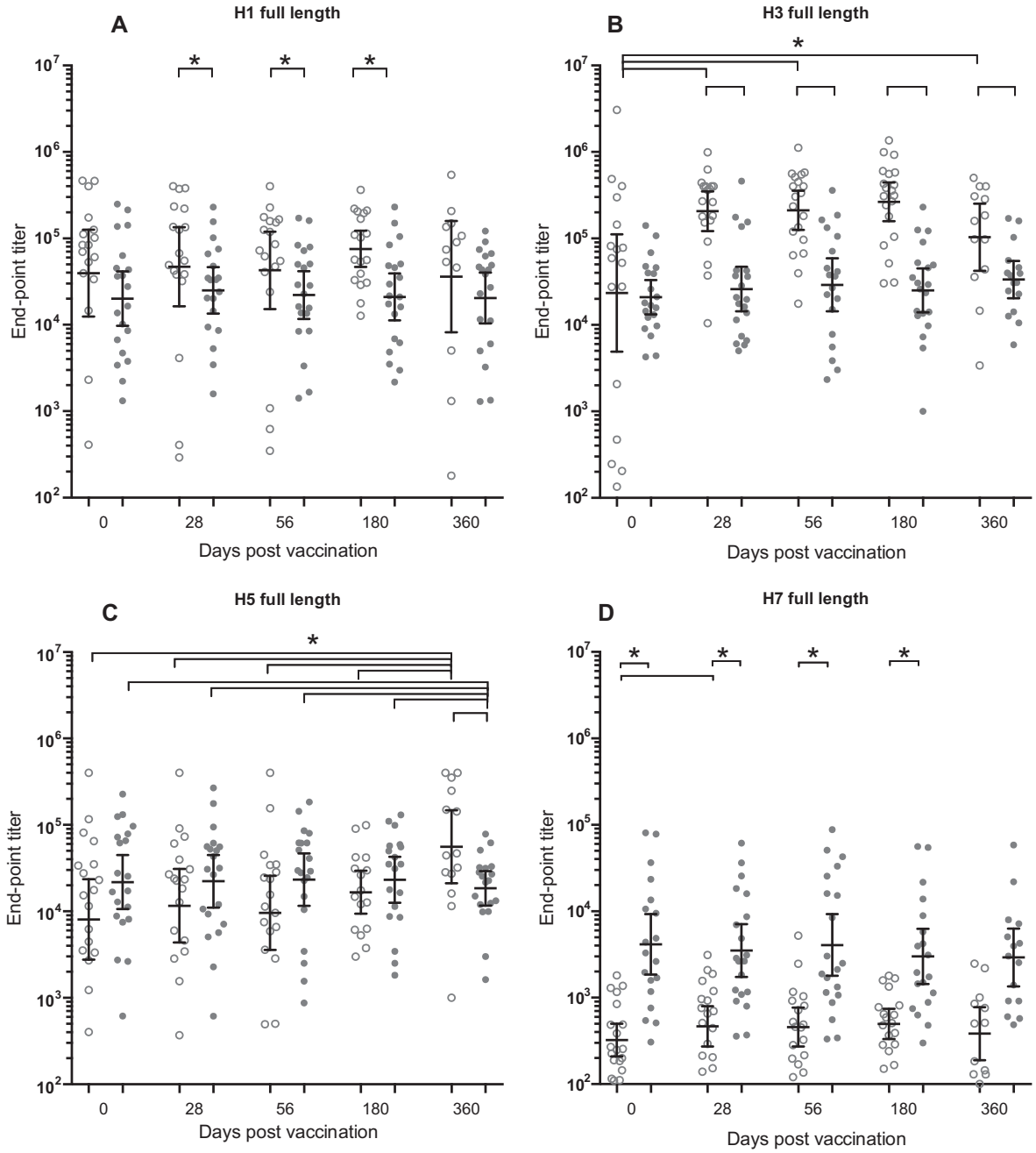

Fig. 3. The haemagglutinin specific antibody response to seasonal and heterosubtypic influenza A viruses. The IgG response to the haemagglutinin of the homologous vaccine strains (A/California/4/2009 (H1N1) (A) and A/Victoria/361/11 (H3N2) (B)) and the avian (A/Vietnam/1203/04 (H5N1) (C) and A/Shanghai/1/13 (H7N9) (D)) viruses was measured by ELISA. Each circle (open = children, filled = adults) represents the endpoint titre of one individual, and the bars show the geometric mean titre ± 95% confidence interval. The sampling points were at day 0 (pre-vaccination) and days 28, 56, 180 and 360-post vaccination. The children and adult sampling points were compared using paired student's *t*-test and *t*-test with Welch correction. $^{*}p < 0.05$.

intranasal LAIV3 vaccination in children and adults. Our results show that LAIV3 boosted the H3-specific antibody responses in children, but not adults, and the antibody response was dominated by antibodies to the HA head domain. A trend of increase in H1 stalk specific antibodies was found in children after LAIV3. Adults who have previously experienced repeated influenza infection had higher pre-existing H1 stalk-specific antibodies, but these were not boosted after LAIV3 immunization.

The golden standard haemagglutination inhibition (HI) assay mainly measures neutralising HA head-specific antibodies with a serum HI titre of 40 considered protective in adults [18]. Although in children HI titres of 110 have been proposed as providing 50% protection to H3N2 after IIV vaccination [19]. We found that LAIV3 only boosted H3N2 HI antibodies (>110) in the children, and these titres were maintained up to a year. Generally, no boost in the HI antibody was observed to the H1N1pdm09 virus in either adults or children, in agreement with our previous findings in children [4]. Pre-existing influenza-specific antibodies, particularly to H1N1pdm09 from previous infection or vaccination, may limit replication of the H1N1pdm09 LAIV strain and therefore restrict stimulation of the immune response [20], but should also provide protection against H1N1pdm09 infection. Interestingly, studies in the USA have shown reduced vaccine effectiveness post-pandemic against H1N1pdm09 after LAIV [21,22] although

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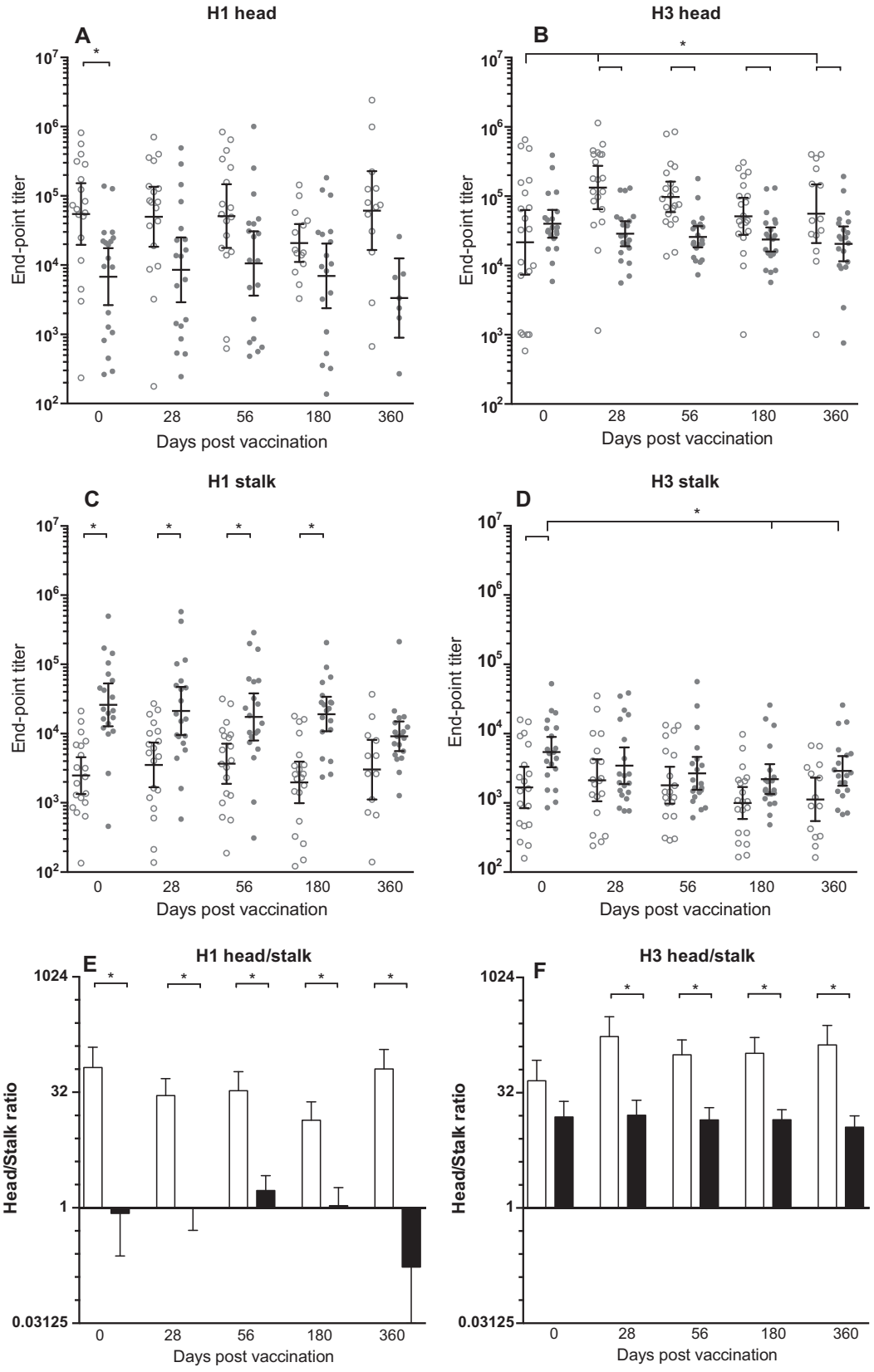

Fig. 4. The IgG HA specific response to the haemagglutinin head and stalk of influenza A H1N1 and H3N2 viruses. The head and stalk IgG responses to the HA of A/California/4/2009 (H1N1) (A, C) and A/Victoria/361/11 (H3N2) (B, D) were measured by ELISA. The time points for sampling were day 0 (pre-vaccination) and days 28, 56, 180 and 360 post vaccination, as indicated on the x-axis. Each circle (open = children, filled = adults) represents the endpoint titre of one individual and the bars show the geometric mean titre ± 95% confidence interval. E (A/California/4/2009 (H1N1)) and F (A/Victoria/361/11 (H3N2)) show the ratio between head and stalk antibody titres on a $\log_2$ scale, where a ratio of 1 shows an equivalent distribution of stalk and head antibody. The bar charts indicate the mean titre ± 95% confidence interval. The children and adult sampling points were compared using paired student's *t*-test and *t*-test with Welch correction. $^{*}p < 0.05$.

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in Europe the LAIV has been shown to be effective against laboratory confirmed influenza [23]. The H1Npdm09 LAIV strain used in 2009–2014 had a mutation in the HA (E47 amino acid residue) that potentially led to reduced thermal stability of this strain [21]. As a consequence of lower effectiveness against H1N1, the LAIV is not recommended in the USA by the Advisory Committee on Immunization Practices (ACIP) for the 2016–17 season although in Europe LAIV is still recommended. These findings of no or low increases in H1N1-specific antibodies observed in our study may help explain the lower effectiveness of the H1N1 strain.

We further used the HA proteins from the homologous vaccine strains to dissect the IgG response to the immunodominant head and the subdominant stalk domains. Our data demonstrate increased IgG antibodies after the first dose of LAIV3 in children to the H3 full-length protein and the H3 head, which persisted up to one year. These head-specific antibodies, that can also be detected by the HI assay, have higher neutralizing capacity than stalk-specific antibodies *in vitro* [24]. Furthermore, our recent study reported that the IgG1 subclass is boosted against the H3 head after LAIV3 [25]. Interestingly the full-length H3N2 HA specific IgG corresponds with the head-specific IgG titres in both groups. The titres of stalk-specific antibodies were determined by age and/or previous exposure history. Adults had higher pre-existing H7 specific antibodies with similar titres to the chimeric H3 stalk. In contrast, the children had low or undetectable levels of pre-existing H7 stalk specific antibodies illustrating the lack of group 2 influenza virus exposure [12]. Lower stalk specific antibodies to the group 2 stalk (H3 and H7) than to group 1 stalk were detected in children, possibly due to exposure to mostly one group 2 virus (H3N2) during their life span.

Both children and adults had pre-existing IgG antibodies to the head of H1, which generally did not boost upon vaccination. Higher head specific titres were observed in children compared to adults, reflecting recent influenza infection with an antigenically similar virus. Interestingly, higher pre-existing stalk H1 antibodies were observed in adults than children probably due to sequential exposure to antigenically distinct HAs from group 1 (seasonal and pandemic H1 and H2 viruses) causing selective boosting of antibodies to the highly-conserved stalk, similarly reviewed by Krammer et al. [10]. The HAs of H5N1 and H1N1pdm09 viruses belong to group 1 with similar stalk, but divergent head domains. Higher levels of H5 antibody were found in adults compared to children pre- and post-vaccination, reflecting adults more extensive previous group 1 infection history resulting in higher levels of cross reactive (heter-subtypic) stalk antibodies. As hypothesized, the presence of a novel globular head in the H1N1pdm09 virus skewed the antibody response to the heterologous stalk in adults [10,26,27]. We have earlier reported that pandemic vaccination with H5N1 vaccine containing a highly divergent HA head and a group 1 stalk boosted the neutralizing stalk-specific responses after the first vaccination in adults [14].

In contrast, children have had limited influenza exposures to group 1 HAs. In agreement, Nachbagauer et al. found that HA stalk-reactive antibodies increased with age after repeated exposure to divergent influenza viruses with conserved stalks [28]. Stalk-reactive antibodies are not extensively induced after seasonal IIV [13,29–32], but we found that children with limited previous influenza infection or earlier pandemic vaccination had pre-existing H1 stalk specific IgG that was boosted by seasonal LAIV3 vaccine, although not significantly. This may allow LAIV3 to be used as a priming strategy for a future universal influenza stalk based vaccine.

The continuous antigenic drift and occasional pandemics with the associated time delay in production of pandemic vaccine highlights the need for development of universal influenza vaccines, which can provide broader and longer lasting immunity. This study shows that LAIV can boost stalk-specific antibodies in children in the absence of a boost in head specific responses a finding, which would need to be confirmed in a larger group of children. Nachbagauer et al. also showed that a high dose recombinant HA seasonal vaccine boosted stalk-specific responses in adults 19–49 years old. Recently, Impagliazzo et al. engineered stable trimeric stems, which were effective at inducing broad protection in pre-clinical animal models [33]. Stalk-specific antibodies provide broader cross reactivity through virus neutralisation and by activation of NK cells through FcγR resulting in lysis of target cells. Further studies could evaluate the functionality of the stalk specific antibodies in children and adults by antibody dependent cellular cytotoxicity (ADCC) and neutralization assays, although a recent study found that LAIV did not induce ADCC in children [34].

In conclusions, adults had higher pre-existing H1 HA stalk antibodies, whereas children had H1 head dominant antibodies probably reflecting recent infection with the same H1 strain. LAIV3 mimics natural infection in children eliciting H3N2 HA head and low levels of stalk H1N1 antibodies, confirming LAIV as a priming of young children.

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Conflict of interest

None.

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References

[1] Longini IM, Koopman JS, Monto AS, Fox JP. Estimating Household and Community Transmission Parameters for Influenza. Am J Epidemiol. 1982;115:736–51.
[2] Viboud C, Boelle PY, Cauchemez S, Lavenu A, Valleron AJ, Flahault A, et al. Risk factors of influenza transmission in households. Brit J Gen Pract 2004;54:684–9.
[3] Fleming DM, Cross KW. Respiratory syncytial virus or influenza? Lancet 1993;342:1507–10.
[4] Mohn KG, Bredholt G, Brokstad KA, Pathirana RD, Aarstad HJ, Tondel C, et al. Longevity of B-cell and T-cell responses after live attenuated influenza vaccination in children. J Infect Dis 2015;211:1541–9.
[5] Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. Lancet Infect Dis 2012;12:36–44.
[6] Block SL, Heikkinen T, Toback SL, Zheng W, Ambrose CS. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. Pediatr Infect Dis J 2011;30:203–7.
[7] Ambrose CS, Wu X, Belshe RB. The efficacy of live attenuated and inactivated influenza vaccines in children as a function of time postvaccination. Pediatr Infect Dis J 2010;29:806–11.
[8] Belshe RB, Edwards KM, Vesikari T, Black SV, Walker RE, Hultquist M, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med 2007;356:685–96.
[9] Belshe RB, Gruber WC, Mendelman PM, Mehta HB, Mahmood K, Reisinger K, et al. Correlates of immune protection induced by live, attenuated, cold-

adapted, trivalent, intranasal influenza virus vaccine. J Infect Dis 2000;181:1133–7.
[10] Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. Curr Opin Virol 2013;3:521–30.
[11] Bullough PA, Hughson FM, Skehel JJ, Wiley DC. Structure of influenza haemagglutinin at the pH of membrane fusion. Nature 1994;371:37–43.
[12] Henry Dunand CJ, Leon PE, Kaur K, Tan GS, Zheng NY, Andrews S, et al. Preexisting human antibodies neutralize recently emerged H7N9 influenza strains. J Clin Invest 2015;125:1255–68.
[13] Ellebedy AH, Krammer F, Li GM, Miller MS, Chiu C, Wrammert J, et al. Induction of broadly cross-reactive antibody responses to the influenza HA stem region following H5N1 vaccination in humans. Proc Natl Acad Sci USA 2014;111:13133–8.
[14] Nachbagauer R, Wohlbold TJ, Hirsh A, Hai R, Sjursen H, Palese P, et al. Induction of broadly reactive anti-hemagglutinin stalk antibodies by an H5N1 vaccine in humans. J Virol 2014;88:13260–8.
[15] Krammer F, Pica N, Hai R, Margine I, Palese P. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. J Virol 2013;87:6542–50.
[16] Pica N, Hai R, Krammer F, Wang TT, Maamary J, Eggink D, et al. Hemagglutinin stalk antibodies elicited by the 2009 pandemic influenza virus as a mechanism for the extinction of seasonal H1N1 viruses. Proc Natl Acad Sci USA 2012;109:2573–8.
[17] S. Tete, F. Krammer, S. Lartey et al. Dissecting the hemagglutinin head and stalk-specific IgG antibody response in healthcare workers following pandemic H1N1 vaccination. http://dx.doi.org/10.1038/npjvaccines.2016.1.
[18] Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond) 1972;70:767–77.
[19] Black S, Nicolay U, Vesikari T, Knuf M, Del Giudice G, Della Cioppa G, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. Pediatr Infect Dis J 2011;30:1081–5.
[20] Ilyushina NA, Haynes BC, Hoen AG, Khalenkov AM, Housman ML, Brown EP, et al. Live attenuated and inactivated influenza vaccines in children. J Infect Dis 2015;211:352–60.
[21] Caspard H, Coelingh KL, Mallory RM, Ambrose CS. Association of vaccine handling conditions with effectiveness of live attenuated influenza vaccine against H1N1pdm09 viruses in the United States. Vaccine 2016;34:5066–72.
[22] https://www.cdc.gov/flu/news/nasal-spray-effectiveness.htm.
[23] Pebody R, Sile B, Warburton F, Sinnathamby M, Tsang C, Zhao H, et al. Live attenuated influenza vaccine effectiveness against hospitalisation due to laboratory-confirmed influenza in children two to six years of age in England in the 2015/16 season. Eurosurveillance 2017;22:18–22.
[24] He W, Mullarkey CE, Duty JA, Moran TM, Palese P, Miller MS. Broadly neutralizing anti-influenza virus antibodies: enhancement of neutralizing potency in polyclonal mixtures and IgA backbones. J Virol 2015;89:3610–8.
[25] Manenti A, Tete SM, Mohn KG, Jul-Larsen A, Gianchecchi E, Montomoli E, et al. Comparative analysis of influenza A(H3N2) virus hemagglutinin specific IgG subclass and IgA responses in children and adults after influenza vaccination. Vaccine 2017;35:191–8.
[26] Sangster MY, Baer J, Santiago FW, Fitzgerald T, Ilyushina NA, Sundararajan A, et al. B cell response and hemagglutinin stalk-reactive antibody production in different age cohorts following 2009 H1N1 influenza virus vaccination. Clin Vaccine Immunol 2013;20:867–76.
[27] Thomson CA, Wang Y, Jackson LM, Olson M, Wang W, Liavonchanka A, et al. Pandemic H1N1 influenza infection and vaccination in humans induces cross-protective antibodies that target the hemagglutinin stem. Front Immunol 2012;3.
[28] Nachbagauer R, Choi A, Izikson R, Cox MM, Palese P, Krammer F. Age dependence and isotype specificity of influenza virus hemagglutinin stalk-reactive antibodies in humans. MBio 2016;7:e01996–e2015.
[29] Wrammert J, Smith K, Miller J, Langley WA, Kokko K, Larsen C, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. Nature 2008;453:667–71.
[30] Suzuki Y. Selecting vaccine strains for H3N2 human influenza A virus. Meta Gene. 2015;4:64–72.
[31] Andrews SF, Huang Y, Kaur K, Popova LI, Ho IY, Pauli NT, et al. Immune history profoundly affects broadly protective B cell responses to influenza. Sci Transl Med. 2015;7:316ra192.
[32] Margine I, Hai R, Albrecht RA, Obermoser G, Harrod AC, Banchereau J, et al. H3N2 influenza virus infection induces broadly reactive hemagglutinin stalk antibodies in humans and mice. J Virol 2013;87:4728–37.
[33] Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science 2015;349:1301–6.
[34] Jegaskanda S, Luke C, Hickman HD, Sangster MY, Wieland-Alter WF, McBride JM, et al. Generation and protective ability of influenza virus-specific antibody-dependent cellular cytotoxicity in humans elicited by vaccination, natural infection, and experimental challenge. J Infect Dis 2016;214:945–52.

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