Neutralizing Antibody Responses to Homologous and Heterologous H1 and H3 Influenza A Strains After Vaccination With Inactivated Trivalent Influenza Vaccine Vary With Age and Prior-year Vaccination

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Background. Prior influenza immunity influences the homologous neutralizing antibody responses elicited by inactivated influenza vaccines (IIV), but neutralizing antibody responses to heterologous strains have not been extensively characterized.

Methods. We analyzed neutralizing antibody titers in individuals aged 1–88 who received the 2009–2010 season IIV before infection by or vaccination against the 2009 pandemic H1N1 virus. Neutralization titers to homologous and heterologous recent, and advanced H1 and H3 strains, as well as H2, H5, and H7 strains, were measured using influenza hemagglutinin pseudoviruses. We performed exploratory analyses based on age, prior-year IIV, and prevaccination titer, without controlling for Type I errors.

Results. IIV elicited neutralizing antibodies to past and advanced H1 and H3 strains, as well as to an H2 strain in individuals who were likely infected early in life. The neutralization of avian subtype viruses was rare, and there was no imprinting of neutralization responses to novel avian subtype viruses based on the influenza group. Compared to adults, children had higher seroresponse rates to homologous and heterologous strains, and their sera generated larger antigenic distances among strains. Seroresponse rates to homologous and heterologous strains were lower in subjects vaccinated with prior-year IIV, though postimmunization titers were generally high.

Conclusions. IIV elicited neutralizing antibodies to heterologous H1 and H3 strains in all ages groups, but titers and seroresponse rates were usually higher in children. Prior-year vaccination with the same strains tended to blunt IIV neutralization responses to all strains in young and old age groups, yet postimmunization titers were high.

Keywords. influenza vaccines; influenza immunity; neutralizing antibodies; hemagglutinin antibodies.

Influenza hemagglutinin (HA) antibodies to variable regions surrounding the receptor binding site in the HA head can block receptor binding and inhibit hemagglutination (H1). H1 antibodies correlate with protection against influenza, but typically neutralize only homologous (matched) influenza strains. Cross-neutralizing antibodies to conserved epitopes outside the receptor binding site, including those in the HA stem, may contribute to protection to homologous and heterologous strains, but the extent to which they are elicited by IIV remains poorly understood.

Studies have shown that both preexisting immunity and age affect antibody responses to IIV, but study parameters and findings have varied [1–10]. Adults often have higher neutralization titers before vaccination, compared to children, due to prior infections or immunizations. The anamnestic recall of antibodies cross-reactive to the virus from an individual's first influenza infection, termed "original antigenic sin" [11, 12], can shape responses. The boosting of antibody responses to the same subtype viruses from past infections has been described as back boosting, and one study showed that antigenically-advanced strains induce antibodies to contemporary strains [13]. Another report suggested that hemagglutinin imprinting from an individual's first influenza infection confers lifelong protection against severe disease from novel hemagglutinin subtypes in the same phylogenetic group [14]. On the other hand, the negative effects of immunological imprinting from earlier exposures, called negative interference or negative antigenic interaction, has also been described [15, 16].

Seasonal IIV can protect against severe disease, but the benefits of annual vaccination are being debated. Some studies show that repeated vaccination has been effective over many years [17, 18], but others indicate no long-term advantage of
annual revaccination [19] or have shown the greatest protection in individuals not vaccinated in prior years [20]. Reduced seroresponses after repeated vaccinations have been noted [3, 21–24]. The receipt of prior IIV has been associated with sustained, higher H1 antibody titers 1 year later, but lower antibody and effector B-cell responses after a new vaccination [3, 25]. Preexisting antibody levels have also been negatively correlated with boosting to the same strain [3, 4, 13], though subjects with high cross-reactive prevaccination neutralization titers often have high postvaccination cross-reactive titers [7].

Here, we analyzed heterologous, neutralizing antibody responses in pediatric and adult subjects who received the 2009–2010 season trivalent IIV prior to either infection by or vaccination against the pandemic 2009 H1N1 virus, to assess how the breadth and level of preimmunization neutralization titers affect the breadth and levels of postimmunization neutralization titers in those with and without a prior-year IIV. We measured neutralizing antibodies to recent and past seasonal viruses, as well as avian and pandemic H2 and H3 viruses, using pseudoviruses bearing HA on their surface (HA-pseudoviruses). Previously, we showed that HA-pseudovirus neutralization titers correlate well with microneutralization titers [26]. Microneutralization titers >160 and a 4-fold increase after vaccination have been proposed as correlates of protection [27]. Therefore, we used a titer of 160 as a seropositive threshold for HA-pseudovirus neutralization. We found that IIV elicits heterologous, neutralizing antibodies to a range of seasonal H3 and H1 HA and that responses varied with both age and vaccination with the prior IIV. These findings offer new insights into how preexisting immunity shapes IIV-elicited neutralization responses to heterologous strains.

**METHODS**

H1, H2, H3, H5, and H7 HA (Table 1) were used to construct HA-pseudoviruses and tested for sera neutralization, as previously described [26, 28, 29] (see Supplementary Materials for details). Subjects and serum samples are summarized in Table 2 (see Supplementary Materials for details). For antigenic maps, see the Supplementary Materials.

**Antibody Landscapes**

Antibody landscape-inspired figures [13] were created by plotting antigenic distances among H3/N2 or H1/N1 viruses, as measured in the ferret antigenic maps (Figure 1), on the x-axis, and plotting pre and postvaccination antibody geometric mean titers (GMT), with 95% confidence intervals to each virus, for each age group, on the y-axis. Plots were made in R 3.3.2 (R Foundation for Statistical Computing).

**Statistical Analysis**

Neutralization titer correlations were evaluated for nonparametric correlations with Spearman’s test. We analyzed 2 sample comparisons and geometric mean titers (GMT) with 95% confidence intervals using GraphPad Prism software.

**Ethics Statement**

The Food and Drug Administration’s Research Involving Human Subjects Committee approved the use of preexisting, de-identified sera as exempt research (Protocol #09-043B), as described under 45 CFR 46.101(b)(4).

**RESULTS**

**Inactivated Influenza Vaccine Elicits Neutralizing Antibodies to Heterologous H3 Influenza Hemagglutinin**

To investigate the breadth of neutralizing antibodies elicited by IIV, we first measured neutralization titers to homologous (vaccine-matched) and heterologous (mismatched) H3 HA in pre and postvaccination sera from subjects aged 2–17 and 25–88 years (Supplementary Figure S1A and S1B) for the following strains: A/Philippines/2/1982 (PH/2/82), a distant-past strain used in 1983–1986 IIVs; A/Wyoming/03/2003 (WY/03/03), a recent-past strain used in the 2004–2005 IIV; A/Bratislava/10/2007 (BR/10/07), a homologous (vaccine-matched) strain used in 2008–2010 IIVs; and A/Victoria/361/2011 (VI/361/11), an advanced (future) strain used in 2012–2014 IIVs (Supplementary Table S1). These H3 HAs are genetically closely related, especially the HAs of WY/03/03, BR/10/07, and VI/361/11 (Figure 1A, left panel). Antigenic cartography using convalescent ferret antisera generated antigenic distances that reflected genetic distances (Figure 1B, left panel, and Supplementary Table S2).

Preexisting neutralization GMT varied with age, in agreement with potential past exposures (Figure 2A). Children ≤17 years,

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Abbreviation: GISAID, Global Initiative on Sharing All Influenza Data; HA, influenza hemagglutinin.

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and therefore not exposed to PH/2/82, had the lowest neutralization GMT to this strain. Similarly, neutralization GMT against the advanced VI/361/11 strain were low (<160) for all age groups. Remarkably, postvaccination neutralization GMT to all strains were ≥160 (Figure 2A). Seroresponse rates (percentage of subjects with a prevaccination neutralization titer <160 and postvaccination neutralization titer ≥160 or with a prevaccination neutralization titer ≥160 and a minimum 4-fold increase in

Table 2. Subject Information by Study Group

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Ages of subjects are provided based on the time of the trials, conducted in 2009.

Figure 1. Genetic and antigenic distances of H3N2 and H1N1 HAs. (A) Phylogenetic trees were constructed for HA from 18 H3N2 influenza A vaccine strains (left panel) and from 9 H1N1 influenza A vaccine strains (right panel). The strains used in this study are colored. (B) Antigenic maps were constructed for H3N2 HA (left panel) and H1N1 HA (right panel), with neutralization titers of convalescent ferret antisera of H1N1 (BJ/262/95, NC/20/99, BR/59/07, and CA/07/09) and H3N2 (PH/2/82, WY/03/03, BR/10/07, and VI/361/11) vaccine viruses against a panel of antigens (H1 HA: BJ/262/95, NC/20/99, BR/59/07, and CA/07/09; H3 HA: PH/2/82, WY/03/03, BR/10/07, and VI/361/11). Abbreviation: HA, influenza hemagglutinin.
postvaccination neutralization titer) to all strains were generally highest in children aged 2–8 years, and the percentage of subjects with titers ≥160 were high for all strains, in all age groups (Supplementary Figure S2). For strains to which subjects were not exposed (PH/2/82 for children and VI/361/11 for all subjects), seroresponse rates were >50%, indicating that IIV induces cross-neutralizing antibodies to heterologous strains of the same subtype (Supplementary Figure S2A). All subjects older than 5 years had potential exposures to WY/03/03 and BR/10/07, but postvaccination titers were generally lower in older subjects (Figure 2A and Supplementary Figure S2A).

Overall, preexisting, neutralizing antibodies to past H3 viruses among those potentially exposed (ages ≥24 years) were prevalent and modestly boosted by IIV (Figure 3A). Notably, IIV increased responses to strains not encountered (PH/2/82 for subjects aged 2–8 and antigenically-advanced strains for all age groups), indicating boosting of cross-neutralizing antibodies.

Inactivated Influenza Vaccine Elicits Neutralizing Antibodies to Heterologous H1 Subtype Influenza Hemagglutinin

We next evaluated the neutralization of H1 strains: A/Beijing/262/1995 (BJ/262/95), a distant-past strain used in the 1998–2000 IIVs; A/New Caledonia/20/1999 (NC/20/99), a recent-past strain used in 2001–2007 IIVs; A/Brisbane/59/2007 (BR/59/07), a homologous strain used in 2008–2010 IIVs; and A/California/07/2009 (CA/07/09), an advanced strain used in 2010–2017 IIVs (Supplementary Table S1 and Supplementary Figure S1C and S1D). BJ/262/95, NC/20/99, and BR/59/07 HAs are genetically close, but CA/07/09 HA is distantly related (Figure 1A, right panel). Convalescent ferret antisera gave a pattern of antigenic

![Figure 2](https://academic.oup.com/cid/advance-article-abstract/doi/10.1093/cid/ciy818/5106972)}

Figure 2. IIV vaccination elicits and boosts neutralizing antibodies’ production of H1 and H3 HA. (A) Neutralization titers to PH/2/82, WY/03/03, BR/10/07, and VI/361/11 HA-pseudoviruses in the sera of different age groups, collected pre- and post-IIV vaccination. (B) Neutralization titers to BJ/262/95, NC/20/99, BR/59/07, and CA/07/09 HA-pseudoviruses in the sera of different age groups, collected pre- and post-IIV vaccination. The GMT with 95% CI are shown. The dotted lines represent the neutralization titer of 160. The gray bar indicates the GMT of prevaccination; the colored bar indicates the GMT of postvaccination. The vaccine strains are indicated in red. Abbreviations: CI, confidence interval; GMT, geometric means of titers; HA, influenza hemagglutinin; IIV, inactivated influenza vaccines.
relatedness consistent with genetic distances (Figure 1B, right panel, and Supplementary Table S2).

Preexisting neutralization GMT were ≥160 for BJ/262/95, NC/20/99, and BR/59/07 in all age groups and for CA/07/09 in adults (Figures 2B and 3B). Interestingly, this included subjects (aged 1–8 years) born after BJ/262/95 circulation, suggesting the presence of cross-neutralizing antibodies. IIV induced neutralizing antibody responses, not only to the BR/59/07 homologous strain, but also to the BJ/262/95 distant-past strain, NC/20/99 recent-past strain, and CA/07/09 advanced-pandemic strain in both children and adults, though boosting was generally lower in adults (Figures 2B and 3B). Seroresponse rates to BJ/262/95 tended to be higher in children, who were less likely to have been exposed, and rates against all strains tended to be lower among the elderly, but the rates of titers ≥160 were high against all strains in adults after IIV (Supplementary Figure S2). Notably, the elderly only exhibited higher neutralization titers compared to the younger for CA/07/09, likely explained by exposures or vaccinations to swine-like influenzas in 1918–1927, 1947–1956, and 1976–1977.

Figure 3. Antibody landscape–inspired figures showing IIV vaccination responses by age group. (A) Neutralizing antibody titers pre and postvaccination for each age group are plotted against PH/2/82, WY/03/03, BR/10/07, and VI/361/11 H3 HA-pseudoviruses. Viruses are plotted by antigenic distance (indicated by arrows) from the vaccine antigen (BR/10/07). (B) Neutralizing antibody titers pre and postvaccination for each age group are plotted against BJ/262/95, NC/20/99, BR/59/07, and CA/07/09 H1 HA-pseudoviruses. The viruses are plotted by antigenic distance (indicated by arrows) from the vaccine antigen (BR/59/07). Antigenic distances among either H3 or H1 viruses were measured using ferret antisera (from Figure 1B and Supplementary Table S3). GMT with 95% CI were shown. The post-GMT were overlaid with the pre-GMT. The dotted lines represent the neutralization titer of 160. Gray shading and dotted bars indicate prevaccination; colored shading and black bars indicate postvaccination. The vaccine strains are indicated in red. Abbreviations: CI, confidence interval; GMT, geometric means of titers; HA, influenza hemagglutinin; IIV, inactivated influenza vaccines.

Prior-year Inactivated Influenza Vaccine Affects Neutralization Responses to the Next Inactivated Influenza Vaccine

Since BR/10/07 (H3) and BR/59/07 (H1) were vaccine strains in the 2008–2009 and 2009–2010 IIVs and some subjects received the 2008–2009 IIV, we studied the effect of repeat vaccinations. Regardless of age, seroresponse rates to most H3 and H1 strains were lower in those who had repeat vaccinations, especially in subjects with preexisting titers ≥160 (Figures 4 and 5, Supplementary Figures S3 and S4, and Supplementary Table S3). Subjects with preexisting titers <160 had the largest seroresponse rates to both homologous and heterologous strains after IIV, and postvaccination titers were generally lower in those with prior-year vaccinations compared to those without. GMT changes to most H3 and H1 HAs postvaccination were lower in the youngest and oldest subjects that had a prior-year vaccination and a preexisting titer ≥160. Postvaccination GMT in these subjects was generally lower than those in the youngest and oldest subjects that had not had the prior year IIV but had a preexisting titer ≥160. We noted that several children had high preexisting titers against CA/09. The reason for this is unknown, but may be due to subclinical CA/09 infections prior to study entry in August 2009. High preexisting titers to H3 and H1 vaccine strains may be partly due to prior year immunization or...
infections with the same H1N1 and H3N2 strains. High preexisting titers to BJ/262/95 in children who could not have been exposed to this strain likely reflect cross-neutralization, induced by NC/20/99. The antigenic distance between NC/20/99 and BJ/262/95 is small (Figure 1B), and NC/20/99 circulated during 2000–2007. High pre and postvaccination titers to NC/20/99 may reflect natural or vaccine exposures over several years. Similarly, high preexisting titers to a past H3 HA were also common and may be explained by the relatively close antigenic distance among the seasonal H3 and prior infections and vaccinations. High titers to past strains postvaccination indicate strong back boosting. The fold change in neutralization titers to all intra-subgroup heterologous HA-pseudoviruses correlated with the fold change in neutralization titers to the homologous BR/10/07 H3 (Supplementary Figures S5 and S6) and BR/59/07 H1 (Supplementary Figures S7 and S8) HA-pseudoviruses, regardless of age, especially for strains most closely related to the vaccine strain. However, vaccination with the prior year IIV containing the same strains reduced this correlation in adults, but not in children.

**Influenza Hemagglutinin Antigenic Distances Determined by Neutralization Titers Vary With Age and Prior Inactivated Influenza Vaccination**

Antigenic cartography using HI titers is used to aid vaccine strain selection. Because neutralizing antibodies detect more epitopes than HI antibodies, we explored a geometric
interpretation [30] of pre and postvaccination neutralization titers using antigenic cartography. The errors associated with the positions of sera in antigenic cartography maps were small (between -1 and 1 antigenic units; data not shown), suggesting that the maps provide a good fit of the data and that the geometric interpretation of the data is reliable. Overall, except for the HAs from distant-past PH/2/82 in subjects born after the virus circulation (ages ≤24 years) and the pan-demic CA/07/09, antigenic distances among the H3 (Figure 6 and Supplementary Tables S4 and S5) and H1 (Figure 7 and Supplementary Tables S4 and S5) HAs were small after IIV, regardless of either age or prior-year vaccination. Except for the pandemic CA/07/09 H1 HA, antigenic distances among HAs of the same subtype often decreased postvaccination in all groups, suggesting a boosting of a preexisting immunity that was enriched in antibodies to conserved regions. Interestingly, prevaccination sera in those who received a prior IIV often generated larger antigenic distances among the strains, compared to the postvaccination sera in those with no prior IIV, consistent with waning of IIV-induced antibodies to the conserved regions.

Sera from the <9 years age group generated a smaller decrease in antigenic distances postvaccination, as compared to sera from the older age groups. This probably reflects the lower preexisting immunity in young children, which reduces the back boosting of antibodies to conserved epitopes. The relative antigenic distances

![Figure 5](https://academic.oup.com/cid/advance-article-abstract/doi/10.1093/cid/ciy818/5106972)
between vaccine strains and other strains generated by sera from young children (<9 years) were more similar to the relative antigenic differences generated by reference antisera from ferrets without preexisting immunities (Supplementary Tables S4 and S5).

**Inactivated Influenza Vaccine Elicits Neutralizing Antibodies to Strains Seen Early in Life, But Not to Novel Subtype Influenza Hemagglutinin**

A recent study suggested that the first influenza infection in life confers protection against severe disease from novel avian influenza strains in the same phylogenetic group through HA imprinting [14]. We therefore evaluated preexisting antibodies to avian strains. All subjects, except a few adults, lacked neutralization titers ≥160 against A/Vietnam/1203/2004 (H5, group 1) and A/Shanghai/02/2013 (H7, group 2) HA-pseudoviruses (Figure 8A, top panel). Pre and postvaccination sera from persons born during 1976–1991, when H1N1 and H3N2 viruses (group 1 and 2, respectively) circulated, did not neutralize (titers < 160) H5 A/Vietnam/1203/2004 (group 1) or H7 A/Shanghai/02/2013 (group 2) HA-pseudoviruses (Figure 8A, middle and bottom panels). Similarly, pre and postvaccination sera from persons born during 1957–1967, when H2N2 (group 1) circulated, also failed to neutralize (titers < 160) H5 and H7 HA-pseudoviruses. Altogether, IIV did not induce or boost cross-neutralizing antibody titers ≥160 against H5 and H7 HA.

We also measured neutralization titers against pandemic H2 and H3 HA for persons born during 1957–1967 or 1968–1975, when H2N2 or H3N2 circulated, respectively. IIV boosted neutralization titers to A/Japan/305/1957 (H2) in persons born during 1957–1967 (Figure 8B, top panel) and to A/Aichi/2/1968...
Influenza A Neutralizing Antibodies

(H3) in persons born during 1968–1975 (Figure 8B, bottom panel), consistent with the original antigenic sin hypothesis. Persons born during 1957–1967 generally had high neutralization titers against A/Japan/305/1957 and A/Aichi/2/1968 before vaccination, consistent with prior exposures, and IIV further boosted these titers. Persons born during 1968–1975, who were therefore without H2 exposure, generally had low neutralization titers against A/Japan/305/1957, although a few subjects had high titers that may be due to their birth at the border of the H2 disappearance. Overall, these data show that IIV boosts neutralization titers to viruses that infected persons in early life.

We also re-evaluated the impact of early-life influenza virus exposures on subsequent responses to current vaccine strains. After IIV vaccination, the neutralization titers against the vaccine strains BR/10/07 (H3) and BR/59/07 (H1) were boosted in the groups born during 1968–1975, 1957–1967, and 1921–1956, when H3N2, H2N2, or H1N1 viruses circulated, respectively (Figure 8C), suggesting that after decades, the first influenza exposures in the life do not apparently interfere with neutralization responses to IIV.

DISCUSSION

We examined the effect of age, preexisting neutralization titers, and prior-year IIV immunization in children and adults on the breadth and magnitude of IIV-elicited neutralization responses to a broad range of heterologous HA, including avian subtypes. Neutralization titers measure antibodies to conserved epitopes in the head and stem of HA, in addition to HI antibodies to the receptor binding site. We found that IIV elicited neutralizing antibodies to heterologous HA representing past and advanced H1N1 and H3N2 strains, and boosted cross-neutralizing antibodies to seasonal strains to which persons could not have been exposed. In contrast, the cross-neutralization of HA

![Figure 7](https://academic.oup.com/cid/advance-article-abstract/doi/10.1093/cid/ciy818/5106972/10.1093/cid/ciy818/fig-3)
likely experienced swine-origin–type H1N1 strains early in life, imprinting for novel subtype viruses. Subjects ≥60 years, who post-IIV vaccination, also suggesting the lack of immunological any H2N2 exposure had no H2 neutralization titers pre- and appearance of H2N2 in 1968. However, the subjects without had titers to H2 HA that were boosted by IIV, despite the dis- hypothesis. Many subjects who were likely exposed to H2N2 life, were boosted, in agreement with the original antigenic sin [14].

Neutralizing antibodies do not appear to explain the protection from H5N1 and H7N7 strains was not seen. Thus, preexisting, neutralizing antibodies early in life [14]. Yet, titers to H2 and H3 HAs, representing strains seen early in life, were boosted, in agreement with the original antigenic sin hypothesis. Many subjects who were likely exposed to H2N2 had titers to H2 HA that were boosted by IIV, despite the dis- appearance of H2N2 in 1968. However, the subjects without any H2N2 exposure had no H2 neutralization titers pre- and post-IIV vaccination, also suggesting the lack of immunological imprinting for novel subtype viruses. Subjects ≥60 years, who likely experienced swine-origin–type H1N1 strains early in life, also had titers ≥160 to CA/07/09 HA, which were modestly boosted by IIV. These findings extend the H1 data from others, who showed the back boosting of past strains [13].

We also observed lower seroresponse rates in those who received the prior year IIV and had high preexisting titers. Postvaccination GMT changes to most H3 and H1 strains were lower both in the young and old cases that had the prior year IIV and higher preexisting titers, but differences in the middle age group were less pronounced. The sample numbers in some of these subgroups were small. Because our analyses were exploratory, we did not control for overall Type I errors, so some of the comparisons could be false positives. Neutralization responses to heterologous HA mirrored the responses to homologous

Figure 8. The impact of first-exposure viruses on neutralizing antibody inductions to novel subtype viruses, first-exposure viruses, and current vaccine strain. (A) Neutralization titers to H5 and H7 in the sera collected pre- and post-IIV vaccination in both children and adult groups. The top shows neutralization titers to A/Vietnam/1203/2004 (H5) and A/Shanghai/02/2013 (H7) HA-pseudoviruses in all subjects; the middle shows neutralization titers to A/Vietnam/1203/2004 (H5) HA-pseudoviruses in the subjects with H1 and/or H3 exposure first (born during years 1957–1991), H3 exposure first (born during years 1968–1975), H2 exposure first (born during years 1957–1967), or H1 exposure first (born during years 1921–1956); and the bottom shows neutralization titers to A/Shanghai/02/2013 (H7) HA-pseudoviruses in the subjects with H1 and/or H3, H3, H2, and H1 exposure first. (B) Neutralization titers to first exposure H2 (A/Japan/305/1957; top) and H3 (A/Aichi/2/1968; bottom) in the sera collected pre- and post-IIV vaccination from the subjects with H3 exposure first (born during years 1968–1975) and H2 exposure first (born during years 1957–1967). (C) Neutralization titers to current vaccine strain BR/10/07 (H3; top) and BR/59/07 (H1; bottom) in the sera collected pre- and post-IIV vaccination from the subjects with H3 exposure first (born during years 1968–1975), H2 exposure first (born during years 1957–1967), and H1 exposure first (born during years 1921–1956). The dotted lines in panels A, B, and C represent the neutralization titer of 160. The GMT of neutralization in each group is shown as a short line. The Wilcoxon signed rank test was applied for comparing the paired samples. All neutralization titers were log2 transformed before test. Abbreviations: GMT, geometric means of titers; HA, influenza hemagglutinin; IIV, inactivated influenza vaccines.
Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


