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MAJOR ARTICLE

Effects of Prior Influenza Exposure on Immunogenicity of Influenza Vaccine

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Background. In this study, we investigated the effects of prior influenza exposure on vaccine-elicited humor immune responses to circulating influenza variants.

Method. We randomly selected 360 participants in previous clinical trials stratified by age. Blood samples were collected and tested by hemagglutination-inhibition tests during the 2015–2016 influenza seasons in China. The antigenic map was plotted and antigenic distance was calculated.

Results. Subjects with H1-priming had higher cross-reactive antibodies titers against A/JiangsuTinghu/11019/2015(H3N2) compared with subjects with B-priming did ($P_{adjusted}$ = .038). Subjects with H1-priming also had higher cross-reactive antibodies titers against A/Jiangsu Qinhuai/11059/2015(H3N2) than subjects with both H1 and B priming ($P_{adjusted}$ = .036). Nevertheless, subjects with no H1 and B-priming had higher cross-reactive antibodies titers against A/Jiangsu Qinhuai/11059/2015(H3N2) than subjects with both H1 and B priming ($P_{adjusted}$ = .012). Antigenic distance was well matched with serological results. Moeover, age-specific differences in human postvaccination responses against the identical circulating strain was noted. In addition, children had the most cross-reactive response to both H3N2 and B-yamagata subtypes.

Conclusions. Our results suggest that prior exposure to H1 or B influenza virus may influence cross-reactivity of H3-specific postvaccination responses and consequently could influence the vaccine effectiveness. Our findings also support that there are age-specific differences in human postvaccination responses.

Keywords. cross-reactivity; seasonal influenza; vaccine.

Seasonal flu is a major public health problem. According to World Health Organization (WHO) estimates, the flu annually causes approximately 3 to 5 million cases of severe illness, and 0.29 to 0.65 million respiratory deaths [1]. Currently, vaccination is the most effective strategy to prevent influenza and against epidemics. However, studies have shown that the effectiveness of influenza vaccine was not very satisfying, generally ranging from 40% to 60% [2]. The mismatch between vaccine strains and epidemic strains was frequently observed and considered to be the primary cause of the compromised vaccine effectiveness [3, 4]. In addition, there are other factors that can affect the influenza vaccine effectiveness as well. Some previous studies have shown that pre-existing immunity from prior influenza vaccination or natural exposure to influenza virus might negatively interfere with the performance of current influenza vaccines [5–8]. However, other researchers found opposite results, that is, influenza vaccines would work better in people who had prior exposure [9–12]. Some postulated mechanisms including reactivation of memory B-cells and "original antigenic sin" or "immunologic imprinting" from first influenza exposure have been proposed [13], which may partially explain why background immunity may increase or decrease vaccine effectiveness, depending on antigenic mismatch, and age-related effects or recent vaccination status, but this is still controversial, and we still do not understand what causes the vaccine to lose effectiveness in some age groups and some seasons and to lose effectiveness in the same people over time.

Since 2009, the H1N1 A/California/07/2009-like virus and the B/Brisbane/60/2008-like virus have been recommended by WHO as vaccine prototype viruses in trivalent influenza vaccines (TIVs) or quadrivalent influenza vaccines (QIVs), but the H3 vaccine component has been updated many times, so whether the compositions of H1N1 and B would affect the cross-reactivity of H3 needs to be studied. In addition, previous exposure to the circulating influenza virus may also result in pre-existing antibodies, which might be able to interfere with

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the immune responses of influenza vaccines in following seasons. In this study, we report the impacts of pre-exposure to influenza on the cross-reactivity of seasonal influenza vaccine strains in the 2015–2016 season.

METHODS

Study Design and Participants

From January 2016 to August 2016, a randomized, parallel-controlled, double-blind, noninferiority Phase III clinical trial of a novel QIV was conducted in Lianyungang City, Jiangsu province, China (NCT02710409). A total of 3664 healthy participants ≥3 years of age were enrolled, stratified by age groups of 3–17 years, 18–59 years, and ≥60 years, and then randomly assigned at a ratio of 2:1:1 to receive 1 dose of the experimental QIV, TIV-Victoria, or TIV-Yamagata. Blood samples were taken from each participant before and 28 days after vaccination, which was reported previously [14]. In this study, we randomly selected 360 paired serum samples pre- and postvaccination from 1832 participants who received the experimental QIV, including 120 children (3–17 years), 120 adults (18–59 years), and 120 elderly people (≥60 years). The randomization list of sampling was generated using R software (version 3.3.2). The serum samples were anonymized and approved for use in this study by institutional review board of Jiangsu Provincial Center for Disease Control and Prevention.

Viruses

The experimental QIVs were developed by Jiangsu GDK Biotechnology Co., Ltd., and the vaccine stain components were formulated according to the recommendations of the WHO use for the 2015–2016 northern hemisphere influenza season. Vaccine strains included the following: H1N1 strain (A/California/7/2009)(CA/09e); H3N2 strain (A/Switzerland/9715293/2013)(SWZ/13e)(clade 3C.3a); Type B virus B/Brisbane/60/2008(B/Bris/60)(Victoria lineage)(clade 1A); and B/Phuket/3073/2013 (B/Pht/3073)(Yamagata lineage)(clade 3) [15]. All vaccines were provided in prefilled syringes (0.5 mL), and each subject received 60 μg of hemagglutinin antigen (HA) in total, 15 μg of HA per strain. During the 2015-2016 influenza season, the circulating influenza strains were isolated from influenza patients, who were captured by the influenza monitor system of Jiangsu Provincial Center for Disease Control and Prevention. There were 3 H1N1 strains [(A/Jiangsu Tinghu/SWL144/2016(clade 6B), A/Jiangsu Gaoyou/SWL1118/2016(clade 6B), A/Jiangsu Quanshan/SWL124/2016) (clade 6B)], 4 H3N2 strains [(A/Jiangsu Qinhuai/11059/2015(clade 3C.2a), A/Jiangsu Tinghu/11019/2015(clade 3C.2a), A/Jiangsu Qingpu/11925/2016(clade 3C.2a), A/Jiangsu Haizhou/19/2016(clade 3C.2a)], 1 Victoria B strain [(B/Jiangsu Haizhou/11051/2015(clade 1A)], and 2 Yamagata B strains [(B/Jiangsu Tianning/16/2016(clade 3), B/Jiangsu Nanjing Gulou/14236/2015(clade 3)]. The vaccine strains were all egg-grown, whereas the circulating strains were all cultured from Madin-Darby canine kidney (MDCK) cells.

Hemagglutination Inhibition Assay

Hemagglutination Inhibition (HAI) titers in serum against the vaccine strains were measured before and after vaccination of experimental QIVs. In addition, the HAI titers postvaccination against the circulating influenza strains were also measured. Serum was preprocessed with receptor-destroying enzyme (Denka-Seiken) and consecutively 2-fold diluted from the initial 1:10 dilution. Virus strains were adjusted to 8 HA units/50 μL in phosphate-buffered saline. The HAI assays were performed by trained staff, using 1% turkey red blood cells for H1N1, H3N2, and type B viruses [16, 17]. Hemagglutination inhibition titers were expressed as the reciprocal of the highest serum dilution that resulted in complete HAI. A titer of 5 was assigned if no inhibition was observed at the starting 1:10 serum dilution.

Antigenic Map Construction

For purpose of minimizing the biases caused by low reactor values (HAI titer ≤1:20), low-rank matrix completion was conducted before constructing the antigenic maps (https://sysbio.missouri.edu/software/AntigenMap/about). Each observed HAI titer was normalized by the overall maximum value max(H_{ij}) and the maximum value for each column max(H_j), and the normalized value would be transformed into $N_{ij} = [\max\log_2(H_{ij})]\text{-}\log_2[\max(H_i)/H_{ij}]$ [18]. We used a 2-dimensional map with multidimensional scaling (MDS) based on Euclidean distance to display antigenic distances (AD) between H1N1, H3N2, and type B viruses characterized. Each horizontal gridline and vertical gridline in the map are 1 antigenic unit distance, which is corresponding to a 2-fold difference in HAI titers.

Statistical Analysis

The previous exposure was defined as seroprotective HAI titer of ≥40 before vaccination. For H3N2, according to the prevaccination HAI titers against H1N1 (CA/09e) and type B (B/Bris/60 and B/Pht/3073) vaccine strains, participants were then divided as follows: (1) H1-priming (HAI titer of ≥40 against H1N1 but of <40 against both type B virus); (2) B-priming (HAI titer of ≥40 against either type B virus but of <40 against H1N1 virus); (3) H1/B-priming (HAI titer of ≥40 against H1N1 and either type B virus); and (4) no H1/B-priming (HAI titer of <40 against H1N1 and both type B virus) [19]. A similar classification method was also used for the analysis of previous exposure on H1N1 and B cross-reactivity, with participants divided into 4 categories according to the prevaccination HAI titers against H3N2 (SWZ/13e) and type B (B/Bris/60 and B/Pht/3073) vaccine strains and the prevaccination HAI titers against H1N1 (CA/09e) and H3N2 (SWZ/13e) vaccine strains, respectively.

In this study, the original HAI titer was log transformed before the calculation of geometric mean titers (GMTs). The GMT ratio was defined as HAI $GMT_{varients}$/HAI $GMT_{vaccines}$ × 100%, and GMT reduction was defined as 1 minus GMT ratio. The Kruskal-Wallis rank-sum test was used to compare the difference of GMT ratio across the different exposure groups and age groups. Two-sided hypothesis tests were conducted, and *P* values less than .05 were considered to have statistical significance. When a significant difference was found, a Bonferoni test was used for pairwise comparisons after significant Kruskal-Wallis test, and Bonferoni-adjusted *P* was calculated. All statistical analyses were performed using SPSS software (version 20.0).

RESULTS

Pre- and Postvaccination Geometric Mean Titers Against Different Influenza Strains

Geometric mean titers of antibodies against the different serotypes of influenza vaccine strains and circulating strains were demonstrated in Table 1. For influenza vaccine stains, the GMTs of the antibodies against A/California/7/2009(H1N1), A/Switzerland/9715293/2013(H3N2), B/Brisbane/60/2008(BV), and B/Phuket/3073/2013(BY) were 17.11, 31.87, 11.64, and 21.52 before vaccination and increased to 288.96, 357.81, 74.64, and 172.81 at day 28 after vaccination, respectively. For influenza circulating strains, the postvaccination GMTs of the antibodies against A/Jiangsu Tinghu/SWL144/2016 (H1N1), A/Jiangsu Gaoyou/SWL1118/2016(H1N1), and A/Jiangsu Quanshan/SWL 124/2016(H1N1) were 190.18, 207.95, and 70.24. Postvaccination GMTs of antibodies against A/Jiangsu Qinhuai/11059/2015(H3N2), A/Jiangsu Tinghu/11019/2015(H3N2), A/Jiangsu Qingpu/11925/2016(H3N2), and A/Jiangsu Haizhou/19/2016 (H3N2) were 38.25, 106.22, 93.62, and 133.05, respectively. For BV, postvaccination GMTs of antibodies against B/Jiangsu Haizhou/11051/2015 were 68.93. For BY, GMTs of antibodies against B/Jiangsu Tianning/16/2016 and B/Jiangsu Nanjing Gulou/14236/2015 were 93.08 and 83.17.

Effects of Prior Exposure on Hemagglutination Inhibition Assay Cross-Reactivity of 2015/2016 Quadrivalent Influenza Vaccine

The postvaccination responses were distinct in subjects with H1-priming, B-priming, H1/B-priming, or no H1/B-priming (Table 2, Figures 1A and 2A–D). To be specific, subjects with H1-priming had a GMT reduction of 52.04% against A/Jiangsu Tinghu/11019/2015(H3N2) compared with that against SWZ/13e(H3N2), followed by subjects with no

Table 1. Geometric Mean Titers of Antibodies Against the Different Serotypes of Vaccine Strains and Circulating Strains

Strains	3–17	18–59	≥60	Total
Prevaccination GMTs (95% CI)				
Vaccine Strains				
A/California/7/2009	24.34 (20.65–28.68)	15.97 (14.08–18.11)	12.89 (11.76–14.13)	17.11 (15.80–18.53)
A/Switzerland/9715293/2013	45.68 (37.82–55.18)	24.62 (21.22–28.57)	28.78 (24.91–33.25)	31.87 (28.94–35.10)
B/Brisbane/60/2008	11.62 (10.84–12.45)	11.55 (10.83–12.33)	11.76 (11.02–12.54)	11.64 (11.21–12.09)
B/Phuket/3073/2013	21.68 (18.78–25.03)	20.23 (17.86–22.92)	22.71 (19.96–25.83)	21.52 (19.94–23.22)
Postvaccination GMTs (95% CI)				
Vaccine Strains				
A/California/7/2009	457.81 (360.99–580.59)	342.97 (285.93–411.38)	153.66 (116.13–203.32)	288.96 (250.32–333.56)
A/Switzerland/9715293/2013	449.94 (364.29–555.72)	234.25 (194.95–281.48)	434.62 (348.92–541.35)	357.81 (316.87–404.03)
B/Brisbane/60/2008	80.00 (63.44–100.89)	75.51 (64.00–89.08)	68.84 (56.20–84.34)	74.64 (66.49–83.79)
B/Phuket/3073/2013	172.48 (143.44–207.39)	169.51 (142.35–201.86)	176.51 (145.32–214.39)	172.81 (155.49–192.06)
Circulating Strains				
H1N1				
A/Jiangsu Tinghu/SWL144/2016	447.35 (352.35–567.96)	192.56 (139.85–261.18)	80.46 (56.49–114.62)	190.18 (149.92–221.05)
A/Jiangsu Gaoyou/SWL1118/2016	226.27 (168.76–303.39)	305.19 (218.71–425.87)	131.47 (97.96–176.45)	207.95 (165.35–239.27)
A/Jiangsu Quanshan/SWL124/2016	132.23 (102.71–170.24)	69.40 (52.19–92.29)	37.75 (30.91–46.11)	70.24 (58.04–79.20)
H3N2				
A/Jiangsu Qinhuai/11059/2015	33.83 (28.93–39.57)	29.92 (25.72–34.80)	54.96 (45.02–67.09)	38.25 (33.37–41.27)
A/Jiangsu Tinghu/11019/2015	227.58 (187.79–275.81)	106.31 (86.56–130.58)	49.53 (37.93–64.68)	106.22 (88.00–118.61)
A/Jiangsu Qingpu/11925/2016	103.75 (81.94–131.36)	75.85 (64.25–89.54)	103.75 (88.48–121.65)	93.62 (80.18–101.36)
A/Jiangsu Haizhou/19/2016	73.79 (55.53–98.04)	141.28 (114.69–174.05)	226.27 (180.36–283.88)	133.05 (109.65–148.81)
BV				
B/Jiangsu Haizhou/11051/2015	76.39 (59.91–97.39)	68.17 (58.50–79.45)	62.88 (50.51–78.28)	68.93 (58.70–75.44)
BY				
B/Jiangsu Tianning/16/2016	126.26 (101.57–156.96)	115.51 (91.06–146.51)	55.60 (44.19–69.95)	93.08 (77.80–103.26)
B/Jiangsu Nanjing Gulou/14236/2015	102.56 (81.75–128.65)	97.27 (78.28–120.87)	57.89 (46.81–71.59)	83.17 (70.13–91.62)

Abbreviations: CI, confidence interval; GMTs, geometric mean titers.

Table 2. H3 Antigenic Distances for Influenza-Circulating Variants Characterized by Human Postvaccination Sera According to Prior Exposure

	H1-Priming		B-Priming		H1/B-Priming		No H1/B-Priming	
H3N2 Antigenic Distance[a]	Individual	Average	Individual	Average	Individual	Average	Individual	Average
A/Switzerland/9715293/2013 (2015–2016 H3 Vaccine Prototype)								
A/Jiangsu Qinhuai/11059/2015	2.60	1.89 ± 0.69	3.13	2.15 ± 0.60	3.85	2.47 ± 0.82	2.50	1.79 ± 0.48
A/Jiangsu Tinghu/11019/2015	1.01		2.16		1.81		1.88	
A/Jiangsu Qingpu/11925/2016	1.41		1.58		1.87		1.60	
A/Jiangsu Haizhou/19/2016	2.52		1.73		2.33		1.17	

[a]Antigenic distances of individual variants to the vaccine prototype virus were calculated based on the postvaccination hemagglutination inhibition titers. Average distances are shown as mean ± standard error of the mean.

H1/B-priming (GMT reduction of 69.14%), subjects with H1/B-priming (GMT reduction of 73.11%), and subjects with only B-priming (GMT reduction of 77.98%), respectively. In terms of H3-specific antibodies GMT ratios, significant differences between subjects with H1-priming and with only B-priming were observed ($P_{adjusted}$ = .038). Accordingly, A/Jiangsu Tinghu/11019/2015 showed a closer distance to SWZ/13e in the antigenic map derived from H1-priming serum (AD = 1.01) than those derived from no H1/B-priming serum (AD = 1.88), H1/B-priming serum (AD = 1.81), and B-priming serum (AD = 2.16).

A similar pattern of antibodies against A/Jiangsu Qingpu/11925/2016(H3N2) was also observed. Subjects with H1-priming had a numerically less GMT reduction (67.91%) than subjects with B-priming (74.62%), H1/B-priming (75.79%), and no H1/B-priming (74.52%). In the antigenic map, A/Jiangsu Qingpu/11925/2016 showed a closer distance to SWZ/13e in the map derived from H1-priming serum (AD = 1.41) than those derived from only B-priming serum (AD = 1.58), H1/B-priming serum (AD = 1.87), and no H1/B-priming serum (AD = 1.60).

In contrast, subjects with H1-priming had a numerically higher GMT reduction (72.07%) against A/Jiangsu Haizhou/19/2016(H3N2) than subjects with B-priming (63.98%) and no H1/B-priming (56.61%) but comparable to subjects with H1/B-priming (73.11%). No significant difference was observed among the different exposure groups. Accordingly, A/Jiangsu Haizhou/19/2016 showed a relatively longer distance to SWZ/13e in the map derived from H1-priming serum (AD = 2.52) compared with those derived from B-priming serum (AD = 1.73), H1/B-priming serum (AD = 2.33), and no H1/B-priming serum (AD = 1.17).

The cross-reactive antibodies against A/Jiangsu Qinhuai/11059/2015(H3N2) were quite low with GMT reduction and reached almost 90% at the 4 different exposure groups. The GMT reduction was 87.67% in subjects with H1-priming, 91.26% in subjects with B-priming, 93.82% in subjects with H1/B-priming, and 87.40% in subjects with no H1/B-priming, respectively. A significant difference of H3-specific antibodies GMT ratios was observed between subjects with H1-priming and those with H1/B-priming ($P_{adjusted}$ = .036) and also between subjects with H1/B-priming and those with no H1/B-priming ($P_{adjusted}$ = .012). Likewise, A/Jiangsu Qinhuai/11059/2015 showed a much longer distance to SWZ/13e in the antigenic map derived from H1/B-priming serum (AD = 3.85) compared with those derived from only H1-priming serum (AD = 2.60), B-priming serum (AD = 3.13), and no H1/B-priming serum (AD = 2.50). The A/Jiangsu Qinhuai/11059/2015(H3N2) strain was also the farthest away from SWZ/13e compared with the other 3 H3N2-circulating stains (Table 2). We used the same method to conduct the similar explorations on postvaccination responses to H1N1- and B-circulating strains Figure 1B and C, and Supplementary Tables 1 and 2, Supplement Figures 1 and 2. For H1N1, GMT ratios were comparable in prior-exposed subjects (including those with H3-priming, B-priming and H3/B-priming), and, comparing to them, the GMT ratio was a little higher in those unexposed subjects, but no significant difference was observed between various subgroups. For B, all groups had comparable GMT ratios, but no significant difference was observed between various subgroups.

To further mine the data, we stratified it according to age and analyzed the effect of different prior exposure on cross-reactivity in the same age group. However, considering that there is collinearity between age and exposure and the results of age-stratified analysis is similar to the results in the text, we illustrated the findings in the Supplement (Supplementary Figures 3–5).

Effects of Age on Hemagglutination Inhibition Assay Cross-Reactivity of 2015/2016 Quadrivalent Influenza Vaccine

The postvaccination HAI titers for study subjects who had no priming (a prevaccination HAI titer of <40) against vaccine prototype viruses of the 2015–2016 QIVs were used to construct age-specific antigenic maps and calculated the AD separately (Table 3, Figure 3, and Supplementary Figures 6–8).

For H1N1(Table 3, Figure 3, and Supplementary Figure 6), less GMT reduction of antibodies against A/Jiangsu Tinghu/SWL144/2016(H1N1) was observed in children (4.67%) than those in adults (46.80%) and the elderly (16.60%). The difference of H1-specific antibodies GMT ratios between children and adults was significant ($P_{adjusted}$ = .038). Accordingly, A/Jiangsu Tinghu/SWL144/2016 was closer to CA/09e in children

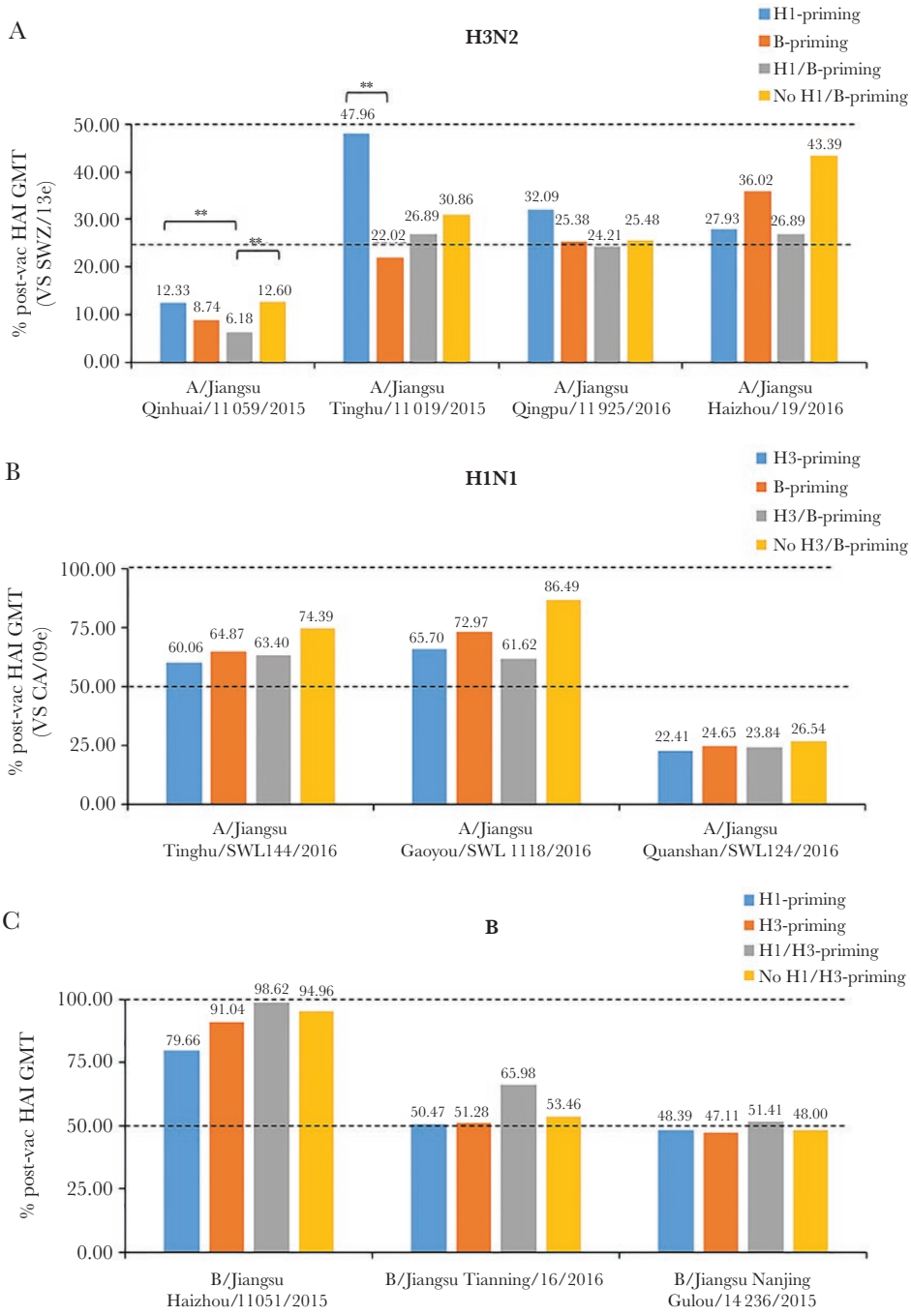

Figure 1. Effects of prior exposure on cross-reactivity of the 2015–2016 Northern Hemisphere influenza vaccines. *A-C*, Postvaccination (post-vac) hemagglutinin inhibition titers against circulating strains during 2015-2016 influenza season are expressed as GMT ratios, which was defined as HAI $GMT_{varients}$/HAI $GMT_{vaccines}$*100%. According to the pre-vaccination HAI titers against H1N1 (CA/09e) and type B (B/Bris/60 and B/Pht/3073) vaccine strains, participants were then divided as follows: (1) H1-priming (HAI titer of ≥40 against H1N1 but of <40 against both type B virus), (2) B-priming (HAI titer of ≥40 against either type B virus but of <40 against H1N1 virus) (3) H1/B-priming (HAI titer of ≥40 against H1N1 and either type B virus), and (4) no H1/B-priming (HAI titer of <40 against H1N1 and both type B virus). The similar classification method was also used for the analysis of previous exposure on H1N1 and B cross-reactivity, with participants divided into four categories according to the pre-vaccination HAI titers against H3N2 (SWZ/13e) and type B (B/Bris/60 and B/Pht/3073) vaccine strains, and the pre-vaccination HAI titers against H1N1 (CA/09e) and H3N2 (SWZ/13e) vaccine strains, respectively. *A*, The H3N2 vaccine trial (50 participants with H1-priming, 93 with B-priming, 34 with H1/B-priming and 183with no H1/B-priming,). *B*, The H1N1 vaccine trial (117 participants with H3-priming, 54 with B-priming, 73 with H3/B-priming and 116 with no H3/B-priming,). C, The B vaccine trial (34 participants with H1-priming, 140 with H3-priming, 50 with H1/H3-priming and 136 with no H1/H3-priming,). The vaccine strains included H1N1 strain (A/California/7/2009)(CA/09e), H3N2 strain (A/Switzerland/9715293/2013) (SWZ/13e), Type B virus B/Brisbane/60/2008(B/Bris/60) and B/Phuket/3073/2013(B/Pht/3073). The circulating influenza strains included three H1N1 strains (A/Jiangsu Tinghu/SWL144/2016, A/Jiangsu Gaoyou/SWL1118/2016, A/Jiangsu Quanshan/SWL124/2016), four H3N2 strains (A/Jiangsu Qinhuai/11059/2015, A/Jiangsu Tinghu/11019/2015, A/Jiangsu Qingpu/11925/2016, A/Jiangsu Haizhou/19/2016), one Victoria B strain (B/Jiangsu Haizhou/11051/2015) and two Yamagata B strains (B/Jiangsu Tianning/16/2016, B/Jiangsu Nanjing Gulou/14236/2015). Kruskal-Wallis rank-sum test was used to compare the difference of GMT ratio across the different exposure groups. Two-sided hypothesis tests were conducted and *P* values less than .05 were considered to have statistical significance. When a significant difference was found, further pairwise comparisons were performed and Bonferoni-adjusted *P* were calculated. ** means $P<.05$.

Figure 2. Effects of prior exposure on antigenic distances of H3N2 circulating variants determined by post-vaccination sera from subjects vaccinated with the 2015–2016 Northern Hemisphere seasonal vaccine. *A-D*, Antigenic maps were constructed using post-vaccination hemagglutinin inhibition titers from healthy participants who had been vaccinated with 1 dose of the 2015-2016 Northern Hemisphere quadrivalent vaccines. *A*, H1-priming (n = 50); *B*, B-priming (n = 93); *C*, H1/B-priming (n = 34); *D*, No H1/B-priming (n = 183). H3 strains in the testing panel including egg-grown A/Switzerland/9715293/2013(SWZ/13e) and cell-grown A/Jiangsu Qinhuai/11059/2015, A/Jiangsu Tinghu/11019/2015, A/Jiangsu Qingpu/11925/2016, A/Jiangsu Haizhou/19/2016. Each gridline (horizontal and vertical) in the maps represents one antigenic unit distance corresponding to a 2-fold difference in HAI titers.

(AD = 0.50) than in adults (AD = 1.35) and the elderly (AD = 0.56) in the antigenic map.

In contrast, antibodies against A/Jiangsu Gaoyou/SWL1118/2016(H1N1) with a more GMT reduction was observed in children (47.55%) than those in adults (6.21%) and the elderly (6.39%), with a significant difference of GMT ratios found between children and adults ($P_{adjusted}$ = .007). The A/Jiangsu Gaoyou/SWL1118/2016 was farther away from CA/09e in children (AD = 0.76) than in adults (AD = 0.22) and the elderly (AD = 0.47) in the antigenic map.

Children had a GMT reduction of 75.59% against A/Jiangsu Quanshan/SWL124/2016(H1N1) compared with that against CA/09e(H1N1), followed by the elderly (GMT reduction of 75.41%) and adults (GMT reduction of 69.83%), respectively, although no significant difference was observed. However, in the antigenic map, A/Jiangsu Quanshan/SWL124/2016 was farther away from CA/09e in the elderly (AD = 3.58) than in children (AD = 1.65) and adults (AD = 1.26).

For H3N2 (Table 3, Figure 3, and Supplementary Figure 7), the elderly had a GMT reduction of 87.24% against A/Jiangsu Tinghu/11019/2015(H3N2) compared with that against SWZ/13e(H3N2), followed by the adults (GMT reduction of 52.15%) and children (GMT reduction of 41.19%), respectively. Significant differences of H3-specific antibodies GMT ratios were observed between children and the elderly ($P_{adjusted}$ = .000), as well as adults and the elderly ($P_{adjusted}$ = .001). Accordingly, in the antigenic map, A/Jiangsu Tinghu/11019/2015 was farther away from SWZ13/e(H3N2) in the elderly (AD = 3.26) than in children (AD = 0.78) and adults (AD = 1.85).

Moreover, adults had a GMT reduction of 84.44% against A/Jiangsu Qinhuai/11059/2015(H3N2) compared with that against SWZ/13e(H3N2), whereas the elderly had a GMT reduction of

Table 3. Antigenic Distances for Influenza Circulating Variants Characterized by Human Postvaccination Sera According to Age

	3–17		18–59		≥60	
H1N1 Antigenic Distance[a]	Individual	Average	Individual	Average	Individual	Average
A/California/7/2009 (2015–2016 H1 Vaccine Prototype)						
A/Jiangsu Tinghu/SWL144/2016	0.50	0.97 ± 0.49	1.35	0.94 ± 0.51	0.56	1.54 ± 1.45
A/Jiangsu Gaoyou/SWL1118/2016	0.76		0.22		0.47	
A/Jiangsu Quanshan/SWL124/2016	1.65		1.26		3.58	
H3N2 Antigenic Distance[a]						
A/Switzerland/9715293/2013 (2015–2016 H3 Vaccine Prototype)						
A/Jiangsu Qinhuai/11059/2015	3.18	2.19 ± 0.94	2.79	1.87 ± 0.66	2.57	2.15 ± 0.87
A/Jiangsu Tinghu/11019/2015	0.78		1.85		3.26	
A/Jiangsu Qingpu/11925/2016	1.93		1.93		1.86	
A/Jiangsu Haizhou/19/2016	2.88		0.93		0.91	
B Antigenic Distance[a]						
B/Brisbane/60/2008 (2015–2016 BV Vaccine Prototype)						
B/Jiangsu Haizhou/11051/2015	0.11	-	0.22	-	1.06	-
B/Phuket/3073/2013(2015–2016 BY Vaccine Prototype)						
B/Jiangsu Tianning/16/2016	0.39	-	1.35	-	1.54	-
B/Jiangsu Nanjing Gulou/14236/2015	0.56		1.12		1.49	

[a]Antigenic distances of individual variants to the vaccine prototype virus were calculated based on the postvaccination hemagglutination inhibition titers. Average distances are shown as mean ± standard error of the mean.

87.75% and children had a GMT reduction of 90.41%, respectively. No significant difference was observed across the groups. However, in the antigenic map, A/Jiangsu Qinhuai/11059/2015 was closer to SWZ/13e in the elderly (AD = 2.57) than in children (AD = 3.18) and adults (AD = 2.79), respectively.

Likewise, antibodies against A/Jiangsu Qingpu/11925/2016(H3N2) and A/Jiangsu Haizhou/19/2016(H3N2) had numerically less GMT reduction in adults than those in the elderly and children. In the antigenic map, A/Jiangsu Qingpu/11925/2016 was closer to SWZ/13e in the elderly (AD = 1.86) than in children (AD = 1.93) and adults (AD = 1.93). The A/Jiangsu Haizhou/19/2016 was closer to SWZ/13e in the elderly (AD = 0.91) than in children (AD = 2.88) and adults (AD = 0.93).

For BV(Table 3, Figure 3, and Supplementary Figure 8), children had a GMT reduction of 7.16% against B/Jiangsu Haizhou/11051/2015 compared with that against B/Bris/60, followed by the elderly (GMT reduction of 4.92%) and adults (GMT reduction of 4.09%), respectively. No significant difference was observed. In addition, in the antigenic map, B/Jiangsu Haizhou/11051/2015 was closer to B/Bris/60 in children (AD = 0.11) than in adults (AD = 0.22) and the elderly (AD = 1.06)

For BY, antibodies against B/Jiangsu Tianning/16/2016(BY) with more GMT reduction was observed in the elderly (66.17%) than those in children (13.80%) and adults (32.81%), with a significant difference of GMT ratios observed between children and the elderly ($P_{adjusted}$ = .001), and a significant difference of GMT ratios also observed in adults and the elderly ($P_{adjusted}$ = .002). Moreover, in the antigenic map, B/Jiangsu Tianning/16/2016 was farther away from B/Pht/3073 in the elderly (AD = 1.54) than in children (AD = 0.39) and adults (AD = 1.35).

A similar pattern of antibodies against B/Jiangsu Nanjing Gulou/14236/2015(BY) with more GMT reduction was observed in the elderly (63.51%) than those in children (32.70%) and adults (46.43%), with a significant difference observed between children and the elderly ($P_{adjusted}$ = .033). In addition, in the antigenic map, B/Jiangsu Nanjing Gulou/14236/2015 was farther away from B/Pht/3073 in the elderly (AD = 1.49) than in children (AD = 0.56) and adults (AD = 1.12).

DISCUSSION

In this study, we analyzed the cross-reactivity of seasonal influenza vaccine strains and explored how pre-exposures have an impact on cross-reactivity used with antigenic mapping. Our results indicate that prior exposure to H1 or B influenza may influence the cross-reactivity of H3-specific postvaccination responses and consequently might influence the vaccine effectiveness. In addition, there is an age-specific difference in human postvaccination responses against the circulating strains, and children may have the most cross-reactive response to both H3N2 and B-yamagata subtypes. Moreover, we conclude that children with initial exposure to H1N1 strains have more restricted response to H3N2 vaccination (Supplementary Figures 9–11).

In this research, we found that protection of vaccine strains against circulating strains were heterogeneous. For H3N2 subtype, the 3C.3a vaccine strain had been a mismatch to the prevalent 3C.2a-circulating viruses, and that could be a reason why most GMT reductions fell by more than 50%. For H1N1 and B subtypes, vaccines provided relatively good protection. In addition, although some circulating strains belonged to the same

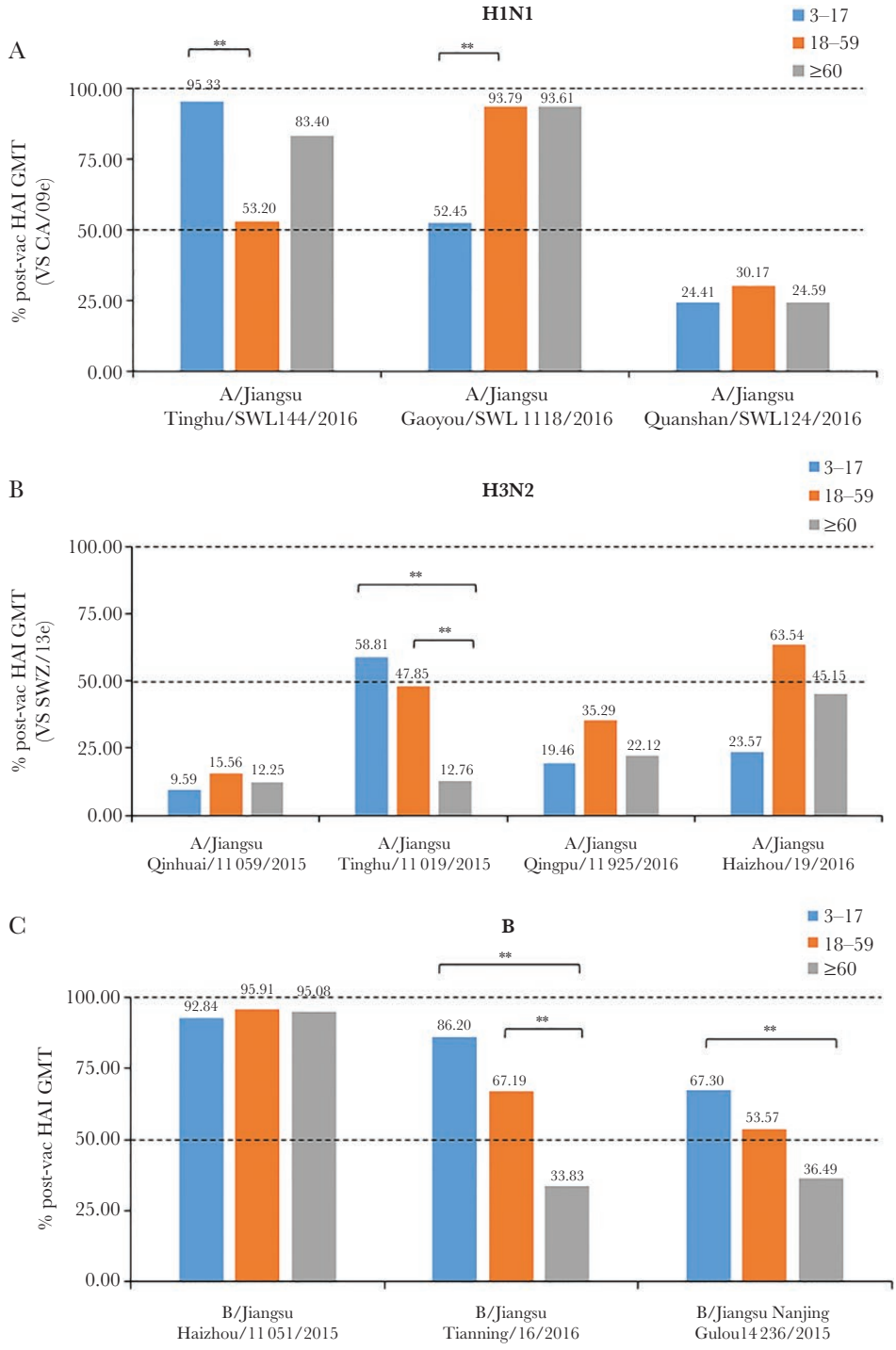

Figure 3. Age effect on cross-reactivity of the 2015–2016 Northern Hemisphere influenza vaccines. *A-C*, Postvaccination (post-vac) hemagglutinin inhibition titers against circulating strains during 2015-2016 influenza season are expressed as GMT ratios, which was defined as HAI $GMT_{varients}$/HAI $GMT_{vaccines}$*100%. The post-vaccination HAI titers for study subjects who had no priming (a pre-vaccination HAI titer of <40) against vaccine prototype viruses of the 2015–2016 QIVs were used. *A*, The H1N1 vaccine trial (29 children aged 3-17 years. 45 adults aged 18-59 years, 42 elderly people aged 60 years or older.). *B*, The H3N2 vaccine trial (47 children aged 3-17 years. 68 adults aged 18-59 years, 68 elderly people aged 60 years or older.). *C*, The B vaccine trial (28 children aged 3-17 years.53 adults aged 18-59 years, 55 elderly people aged 60 years or older.). The vaccine strains included H1N1 strain (A/California/7/2009)(CA/09e), H3N2 strain (A/Switzerland/9715293/2013) (SWZ/13e), Type B virus B/Brisbane/60/2008(B/Bris/60) and B/Phuket/3073/2013(B/Pht/3073). The circulating influenza strains included three H1N1 strains (A/Jiangsu Tinghu/SWL144/2016, A/Jiangsu Gaoyou/SWL1118/2016, A/Jiangsu Quanshan/SWL124 /2016), four H3N2 strains (A/Jiangsu Qinhuai/11059/2015, A/Jiangsu Tinghu/11019/2015, A/Jiangsu Qingpu/11925/2016, A/Jiangsu Haizhou/19/2016), one Victoria B strain (B/Jiangsu Haizhou/11051/2015) and two Yamagata B strains (B/Jiangsu Tianning/16/2016, B/Jiangsu Nanjing Gulou/14236/2015). Kruskal-Wallis rank-sum test was used to compare the difference of GMT ratio across the different exposure groups. Two-sided hypothesis tests were conducted and $P < .05$ were considered to be statistical significant. When a significant difference was found, further pairwise comparisons were performed and Bonferoni-adjusted *P* were calculated. ** means $P < .05$.

branch, mutations occurred at some key sites and consequently changed their original antigenicity; therefore, it was not surprising that GMT levels within the same subtype of circulating strains were so different.

We also found that subjects without priming had higher H3-specific cross-reactive antibodies titers than subjects with H1/B-priming, which was in line with Thompson et al's [8] research that influenza vaccine effectiveness against A(H3N2) virus illness was higher among those unexposed individuals during the previous influenza seasons. We also noted that subjects with H1-priming had higher H3-specific cross-reactive antibodies titers than subjects with B-priming, which is consistent with Xie et al's [19] study, suggesting that previous H1 virus exposure might affect 2015–2016 human postvaccination responses to H3 variants more than previous type B virus exposure. However, the reason why subjects with H1-priming at baseline had H3-specific higher cross-reactive antibodies titers than subjects with H1/B-priming is still unclear and needs future exploration. We also explored how prior exposure to H3N2 and B could affect H1N1 cross-reactivity and prior exposure to H1N1 and H3N2 could affect type B cross-reactivity, but there was no significant difference between various subgroups. It is possible that the H3N2 influenza strains are prone to drift, and the WHO has updated the H3 components several times; therefore, the classification shaped by the WHO could not fully reflect the prior exposure histories. It is also possible that the relatively small variation of H1N1 and B influenza strains provide relatively stable protection. Furthermore, it is possible that there is bad homogeneity in prevaccination titer between strains within each subtype. In this study, we used antigenic maps to calculate the AD, and the results were well matched with serological results, which were mainly manifested as follows: the farther AD is, the more GMT reduction decreases.

There are no universally accepted explanations for this phenomenon, ie, that prior exposure has different effects on cross-reactivity of influenza strains, and the underling mechanism is unknown. A contributing theory is the hypothesis of "original antigenic sin" because it postulates that exposure to influenza antigens could preferentially expand pre-existing memory responses to historical virus antigens at the expense of de novo responses to the current vaccine or infecting strain [20, 21]. Futhermore, although one possible explanation for this phenomenon is that H1 influenza virus commonly causes more serious illness, thus having a greater impact on the immune system [22], the underling mechanism was still unclear and needs future exploration. Moreover, prior exposure is closely related to age. To be more specific, the older you are, the more complex your prior exposure spectrum is, especially for people born before 1957. These people experienced 3 pandemics throughout human history—H2N2 "Asian flu" from 1957 to 1958, H3N2 "Hong Kong flu" in 1968, and H1N1 "swine flu" in 2009 [23]—and what they have experienced is very distinct from children born after 2009. However, at the same time, as people grow older, the immune system changes (immune system hypoplasia in children and immune aging in the elderly), so the impact of prior exposure on the immune responses of the influenza vaccine needs to be considered comprehensively.

Our research has some limitations. First, we did not monitor the influenza incidence rate in the subjects during the following influenza season, thus we can only use cross-reactive antibody levels to estimate the vaccine effectiveness. Second, the vaccine strains were all egg-grown, whereas the circulating variant strains were all cultured from MDCK cells. The manufacturing methods may result in some differences, because the egg-based manufacturing process could introduce antigenically important mutations in high-growth reassortant viruses used for vaccine production [24–26]. Third, we only evaluated the cross-reactivity of influenza vaccine in 1 season; however, a review [27] suggests that vaccine effectiveness might be influenced by vaccination patterns over at least several seasons. Therefore, if possible, we should combine the results of multiple seasons to consider the impacts of prior exposure on vaccine effectiveness.

CONCLUSIONS

In conclusion, we found that prior influenza exposure could have a significant influence on the immune responses of influenza vaccines and the antibodies binding capability against circulating influenza variant strains. The results suggested that a complex pre-existing immunity to previous influenza exposure should also be fully considered in the next generation of influenza vaccine design [28]. In addition, more appropriate animal models may be needed for the influenza vaccine evaluation, because the current naive ferrets' model could not completely reflect the complexity of human exposure history. Therefore, in future vaccination campaigns, the ability to make a more reasonable immunization strategy based on factors such as previous influenza exposure and age is greatly needed.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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