Published in final edited form as:

J Infect Dis. 2021 August 02; 224(3): 469–480. doi:10.1093/infdis/jiaa665.

Impact of Immune Priming, Vaccination, and Infection on Influenza A(H3N2) Antibody Landscapes in Children

Michael Hinojosa[1,2], **Samuel S. Shepard**[1], **Jessie R. Chung**[1], **Jennifer P. King**[3], **Huong Q. McLean**[3], **Brendan Flannery**[1], **Edward A. Belongia**[3], **Min Z. Levine**[1]

[1]Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[2]Oak Ridge Institute for Science and Education, Atlanta, Georgia, USA

[3]Center for Clinical Epidemiology and Population Health, Marshfield Clinic Research Institute, Marshfield, Wisconsin, USA

Abstract

Background.—Preexisting antibodies to influenza, shaped by early infection and subsequent exposures, may impact responses to influenza vaccination.

Methods.—We enrolled 72 children (aged 7–17 years) in 2015–2016; all received inactivated influenza vaccines. Forty-one were also vaccinated in 2014–2015, with 12 becoming infected with A(H3N2) in 2014–2015. Thirty-one children did not have documented influenza exposures in the prior 5 seasons. Sera were collected pre- and postvaccination in both seasons. We constructed antibody landscapes using hemagglutination inhibition antibody titers against 16 A(H3N2) viruses representative of major antigenic clusters that circulated between 1968 and 2015.

Results.—The breadth of the antibody landscapes increased with age. Vaccine-induced antibody responses correlated with boosting of titers to previously encountered antigens. Postvaccination titers were the highest against vaccine antigens rather than the historic A(H3N2) viruses previously encountered. Prevaccination titers to the vaccine were the strongest predictors of postvaccination titers. Responses to vaccine antigens did not differ by likely priming virus. Influenza A(H3N2)-infected children in 2014–2015 had narrower antibody landscapes than those uninfected, but prior season infection status had little effect on antibody landscapes following 2015–2016 vaccination.

Conclusions.—A(H3N2) antibody landscapes in children were largely determined by age-related immune priming, rather than recent vaccination or infection.

Correspondence: Min Z. Levine, PhD, Influenza Division, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta GA 30329 (mlevine@cdc.gov).

Supplementary Data

Supplementary materials are available at *The Journal of 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.

Potential conflicts of interest. H. Q. M. has received research funding from Seqirus, unrelated to the present work. All other authors report no potential conflicts of interest.

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.

Keywords

influenza vaccination; antibody landscape; birth cohorts; immune priming; infection

Human immunity to influenza is complicated by repeated exposures in an individual's lifetime from vaccination and/or natural infection. Vaccination is the most effective public health measure to protect against influenza infections. However, responses to vaccination and influenza vaccine effectiveness are often impacted by multiple factors, including antigenic match between vaccine strain and circulating strains, preexisting immunity from past exposures, and other virological and immunological factors.

Immune responses to influenza vaccination are affected by preexisting antibodies to previously encountered antigens [1–3], including from initial influenza infection and subsequent exposures through vaccination or later infection. Serologic studies since the 1950s have suggested a dominant antibody recall response to the first or early influenza antigen exposure [4–7], referred to by Francis et al [7] as "original antigenic sin" or currently as antigenic seniority [8]. Fonville et al introduced antibody landscapes as a method to visualize serologic data by plotting antibody titers as a function of antigenic relationships among viruses that circulated during different time periods [8]. Recent studies have shown that antibody responses to vaccination can also be modified by prior season vaccinations [9–11]. However thus far, most immune priming and repeated vaccination studies have been conducted in adults whereas data in children remain sparse. In adults, responses to vaccination may be complicated by preexisting immunity from decades of multiple vaccinations and infections. Examination of antibody landscapes among children with well-documented prior exposures may help us to understand factors that can influence vaccine effectiveness.

In this study, we evaluated effects of antibody landscapes on vaccine responses using sera collected from children over 2 influenza seasons in 2014–2015 and 2015–2016. Children were also followed prospectively for influenza infection in both seasons [9]. Antigenic drift of circulating A(H3N2) viruses and a vaccine strain change from 2014–2015 to 2015–2016 (from A/Texas/50/2012 [clade 3C.1] to A/Switzerland/9725193/2013 [clade 3C.3a]) also provided an opportunity to examine effects of infection and vaccine strain changes on antibody landscapes in children. We identified antibody profiles associated with probable immune priming and analyzed effects of prior season vaccination and infection on antibody landscapes.

METHODS

Study Participants and Sera

School-aged children (aged 5–17 years) were enrolled from September to November 2015 in a serologic study in Marshfield, Wisconsin (Figure 1; Supplementary Table 1) [9]. Children included in the antibody landscape analysis (n = 72; age 7–17 years [median age, 13 years]) received 2015–2016 trivalent inactivated influenza vaccine (IIV3, Fluzone, Sanofi Pasteur). Of these, 41 were previously enrolled in 2014–2015 and received either 2014–2015 IIV3

(Sanofi Pasteur) or quadrivalent live attenuated influenza vaccine (LAIV4, FluMist, MedImmune). Children were grouped based on their 2014–2015 A(H3N2) infection and vaccination status: 12 were vaccinated (9 received LAIV and 3 received IIV) and had laboratory-confirmed A(H3N2) infection during the 2014–2015 season (VI group; age 7–13 years [median age, 10.5 years]), 29 were vaccinated and uninfected with A(H3N2) in 2014–2015 (VU group; age 7–17 years [median age, 12 years]), and 31 children were newly enrolled in 2015–2016 from the Marshfield Epidemiologic Study Area who were unvaccinated and uninfected with no documented influenza infection nor medically attended acute respiratory illness in the prior 5 seasons (UU group; age 9–17 years [median age, 13 years]) (Supplementary Table 1) [9]. In 2015–2016, none of the participants had A(H3N2) infection identified during active surveillance. Sera collected prevaccination and 21–28 days postvaccination in 2015–2016 from all children and in 2014–2015 from those enrolled in both seasons were tested.

Written informed consent was obtained from parents/guardians of the children, and assent, when applicable. This study was approved by the institutional review boards of the Centers for Disease Control and Prevention and the Marshfield Research Clinic Institute.

Hemagglutination Inhibition Assays

Vaccine responses were measured by hemagglutination inhibition (HI) assays using 0.5% turkey erythrocytes as previously described [12]. Serum samples were treated with receptor-destroying enzyme and preabsorbed with packed turkey erythrocytes to remove nonspecific agglutinins as needed. Serial 2-fold dilutions of sera were made from an initial 1:20 dilution. The HI titer was defined as the reciprocal of the last dilution of serum that completely inhibited hemagglutination. At least 2 independent experiments were performed for all time points from the same individual with each sample run in duplicates.

All viruses were propagated in 9- to 11-day-old embryonic chicken eggs and sequenced (Supplementary Table 2).

Antibody Landscapes

For each individual, antibody landscapes were constructed for each time point using antibody responses against 16 A(H3N2) viruses representative of antigenic clusters that circulated between 1968 and 2016: A/Aichi/2/1968 (Aichi/1968), A/Victoria/03/1975 (Victoria/1975), A/Bangkok/1/1979 (Bangkok/1979), A/Shanghai/11/1987(Shanghai/1987), A/Beijing/353/1989 (Beijing/1989), A/Beijing/32/1992 (Beijing/1992), A/Wuhan/359/1995 (Wuhan/1995), A/Sydney/05/1997 (Sydney/1997), A/Panama/2007/1999 (Panama/1999), A/Fujian/411/2002 (Fujian/2002), A/California/07/2004 (California/2004), A/Wisconsin/67/2005 (Wisconsin/2005), A/Brisbane/10/2007 (Brisbane/2007), A/Perth/16/2009 (Perth/2009), A/Texas /50/2012 (Texas/2012), and A/Switzerland/9715293/2013 (Switzerland/2013).

To define likely A(H3N2) immune priming, children were grouped into cohorts using 2 approaches. In the first approach, we assumed that the year of birth directly implied priming with a virus that circulated during that year; thus, participants were grouped by birth years as “birth cohorts.” To achieve sufficient numbers per birth cohort, 2 birth years were grouped

as 1 “birth cohort,” except in 2006–2008, where 3 birth years were grouped as 1 birth cohort. To construct antibody landscapes for each birth cohort, we plotted geometric mean titers (GMTs) against viruses arranged chronologically by the year of isolation.

In the second approach, we determined likely priming virus based on an individual’s postvaccination antibody landscape without assuming that initial influenza exposure occurred in the first years of life [13–15], and grouped these children into “priming cohorts.” Likely A(H3N2) priming virus was assumed to be antigenically similar to the earliest reference strain that circulated after a child’s birth with titer ≥40 post 2015–2016 vaccination (Supplementary Tables 3 and 4). Circulation periods for historical A(H3N2) viruses were estimated based on data published for influenza surveillance (Supplementary Table 3). Following the method of Fonville et al [8], we generated an antigenic cartographic map using ferret antisera titers against the 16 A(H3N2) viruses and computed antigenic distance along a “summary path” using R version 3.6.1 software (Supplementary Methods and Supplementary Figure 1). Log-transformed postvaccination titers ($\log_2$ [titer / 5]) were plotted against 16 A(H3N2) reference viruses arranged on the horizontal axis based on the antigenic distance measured from the antigenic map.

Statistical Analysis

GMTs of replicates were reported as final titers. Titers <20 (predilution of sera) were assigned as 10. GMT fold-rise was calculated as the ratio of postvaccination to prevaccination titers. Seroconversion was defined as ≥4-fold rise in antibody titers with postvaccination titers ≥40. Comparison between study groups were analyzed by 2-tailed *t* test or 1-way analysis of variance, and difference in proportions was compared using Fisher exact test. Multivariate linear regression with $\log_2$-transformed titers or fold-rise in titers was used to examine associations between pre- and postvaccination or fold-rise in titers with age and probable priming viruses in both seasons. Statistical analyses were conducted using GraphPad Prism 8.1.1 and SAS 9.4 software.

RESULTS

Impact of Immune Priming

Antibody landscapes in children against 16 A(H3N2) viruses representative of major antigenic clusters that circulated from 1968 to the study period 2016 were compared before and after 2014–2015 and 2015–2016 influenza vaccinations (Figure 2). Vaccination against either Texas/2012 or Switzerland/2013 not only induced antibody rises to the vaccine viruses, it also boosted responses to older viruses, including some that circulated prior to participants’ birth (Figure 2 and Supplementary Figures 2 and 3). Antibody responses in children were broader than those detected by primary infection ferret antisera against the vaccine viruses, which only recognized viruses that circulated in adjacent seasons (Supplementary Table 5).

To investigate the effect of immune priming on vaccine responses, we first grouped the children into 5 birth cohorts that were from birth years 1998–1999, 2000–2001, 2002–2003, 2004–2005, and 2006–2008. At prevaccination, the breadth of antibody landscapes, or the

number of viruses with HI titers ≥40, increased with the age of the birth cohorts (Figure 3 and Supplementary Figure 4A). Children in the same birth cohort, regardless of their prior season vaccination status, had similar shapes of antibody landscapes before vaccination. At baseline, children in the UU group without documented vaccination and infection in the past 5 seasons (n = 31) had lower titers but similar shapes of landscapes to older viruses compared with children who received vaccine in the prior season (VI and VU groups, n = 41), indicating that these children were also exposed to A(H3N2) viruses early in life (Figure 3). Following 2015–2016 vaccination, children in older birth cohorts induced broader antibody responses (back-boost) to earlier viruses; the number of postvaccination titers ≥80 increased with birth cohort years (Supplementary Figure 4B). Of note, young children in the 2006–2008 birth cohort, all of whom received LAIV in 2014–2015, had no detectable HI antibody responses in 2014–2015 (Figure 3).

Next, we used each child's antibody landscape to historic viruses and defined their likely immune priming virus [13]. Antigenic characterization (Supplementary Table 5) and antigenic cartographic map using ferret antisera (Figure 4A) illustrated the gradual and frequent antigenic drift of A(H3N2) viruses over the past 5 decades. Among the children received vaccination in both seasons, we identified 5 priming cohorts. These children were likely primed with Sydney/1997-like, Panama/1999-like, Fujian/2002-like, California/2004-like, and Wisconsin/2005-like A(H3N2) viruses earlier in their life. We constructed antibody cartographic landscapes for these 5 priming cohorts in both seasons (Figure 4B). Similar to the birth cohorts, children primed with older viruses had broader breadth of antibody landscapes at baseline. After vaccination, landscapes based on antigenic distance retained their shapes over 2 seasons within each priming cohort (Figure 4B).

The 2 approaches to identify likely immune priming yielded similar results. In each "birth cohort" or "priming cohort" (Figures 3 and 4), antibody responses were higher to the current vaccine virus and closely related contemporary viruses, rather than to the priming virus or other older viruses they may have been exposed to in the past. In 2015–2016, fold-rise in titers to the likely priming virus was significantly associated with the fold-rise to the vaccine virus Switzerland/2013 ($P < .001$) (Table 1, model 5). However, in the multivariate model, prevaccination titers to the likely priming virus were not significant predictors of responses to vaccine viruses (Table 1, models 1–4). Pairwise comparison of antibody responses to the vaccine viruses in each season among priming cohorts revealed no significant difference ($P > .05$) except that, in the 2014–2015 season, children likely primed with Wisconsin/2005-like viruses (n = 4) had lower responses to Texas/2012 compared to other priming cohorts (Supplementary Tables 6 and 7). Three of the 4 children in this priming cohort received LAIV; lower antibody responses were likely due to the vaccine type received [16], rather than immune priming.

Impact of Prior Season Vaccination

A(H3N2) vaccine antigen was updated from Texas/2012 (3C.1) in 2014–2015 to Switzerland/2013 (3C.3a) in 2015–2016 due to antigenic drift of the circulating viruses. Interestingly, among children enrolled in both seasons (VI and VU groups), following consecutive vaccination with these 2 antigenically distinct antigens, antibody landscape

patterns generally remained similar between seasons (Figure 2A and 2B); similar landscape patterns were also retained within each priming cohort (Figure 3A and 4B). Antibody waning was evident between the postvaccination landscape in 2014–2015 and prevaccination in 2015–2016 (Figures 2–4). In children vaccinated in both seasons, there was a slight increase from 2014–2015 to 2015–2016 prevaccination landscape titers (Figure 5A).

When stratified by prior season vaccination status, at baseline, children unvaccinated and uninfected in the past 5 seasons (UU group, n = 31) had lower 2015–2016 prevaccination landscape titers than those who received 2014–2015 IIV3 (IIV/IIV, n = 29), including titers to the vaccine virus Switzerland/2013 and several viruses that circulated in the earlier influenza seasons (Texas/2012, Perth/2009, Wisconsin/2005, and California/2004; $P < .05$, Figure 5B). However following vaccination with Switzerland/2013, their landscape titers were largely similar ($P > .05$) for all viruses between the 2 groups except for 1 virus (Brisbane/2007) (Figure 5B) where the UU group showed significantly higher titers ($P < .05$). The magnitude of the titers increased but the patterns of the landscapes were retained between pre- and postvaccination in each group. Children in the UU group had significantly higher antibody fold-rise to the vaccine virus Switzerland/2013 (Supplementary Figures 3 and 5) and to the other earlier A(H3N2) viruses compared to children vaccinated in 2014–2015 (IIV/IIV group in Figure 5C and Supplementary Figure 3). Furthermore, in each group, the highest fold-rise was to the vaccine virus Switzerland/2013, rather than to any of the historic A(H3N2) viruses (Figure 5C). Following vaccination in 2015–2016, a greater proportion (94%) of children in the UU group seroconverted to Switzerland/2013 compared with children who received IIV3 in the prior season (63%; $P = .0052$).

In multivariate linear regression models to analyze the predictors for vaccine responses in each season, in 2015–2016, prevaccination titers against vaccine virus Switzerland/2013 were the strongest predictors of postvaccination titers to Switzerland/2013 ($P < .001$; Table 1, model 4). In 2014–2015, prevaccination titers to Texas/2012 were positively correlated with postvaccination titers to Texas/2012 and negatively correlated with fold-rise to Texas/2012 ($P < .05$; Table 1, models 1 and 2).

Impact of Influenza A(H3N2) Infection

Among the 12 children who had reverse-transcription polymerase chain reaction–confirmed infection with A(H3N2) viruses in 2014–2015 (VI group), 9 had received LAIV and 3 received IIV in 2014–2015 (Supplementary Table 1). Circulating A(H3N2) viruses in 2014–2015 were antigenically drifted from the vaccine virus Texas/2012 but were antigenically similar to the updated vaccine strain Switzerland/2013 (3C.3a) in 2015–2016 [16]. Infection with A(H3N2) in 2014–2015 resulted in elevated titers across the landscape in 2015–2016 prevaccination sera, consistent with convalescent response following natural infection. Subsequent vaccination in 2015–2016 further boosted landscape titers (Figure 6A). Conversely, in vaccinated but uninfected children (VU group, n = 29), antibody waned across the landscape from postvaccination in 2014–2015 to prevaccination in 2015–2016, followed by a substantial increase of landscape titers postvaccination with Switzerland/2013 in 2015–2016 (Figure 6B).

Children infected with A(H3N2) after receiving Texas/2012 in 2014–2015 had lower postvaccination titers cross-reactive to Switzerland/2013 compared with those who were vaccinated but not infected ($P = .004$; Supplementary Figure 6A). Postvaccination (preinfection) antibody landscapes among vaccine failure cases (VI) were narrower in breadth and lower in magnitude than those from the vaccinated but uninfected children (VU). Here, postvaccination titers to all 9 recent viruses on the landscape from Sydney/1997 to Switzerland/2013 were significantly lower ($P < .05$) in those who became infected with A(H3N2) than those who were uninfected in 2014–2015 (Figure 5C).

In the following 2015–2016 season, postvaccination antibody landscapes did not differ significantly among the 3 groups of children (Figure 6D; VI, VU, and UU groups; $P > .05$). Children in the UU group had higher fold-rise in titers to most viruses than other groups, likely due to lower prevaccination titers (Supplementary Figure 6B).

DISCUSSION

Many factors can influence an individual's response to influenza vaccination. Examination of A(H3N2) antibody landscapes among children suggested early development of immune priming patterns that were continuously reinforced with new infection and vaccination. Over an individual's lifetime, regular boosting of antibodies against previously encountered antigens can result in increased breadth of antibody landscapes [7, 8]. Recent influenza vaccine studies have suggested that immune priming and birth cohorts can determine an individual's antibody landscape and impact antibody responses to contemporary vaccines, leading to variable vaccine effectiveness [13, 17, 18]. Here, we found that even in young children, immune priming and age are major factors that determine the breadth of the antibody landscape. For each priming cohort, fold-rise in antibody titers to A(H3N2) priming virus was associated with fold-rise to vaccine virus in the 2015–2016 season. Nonetheless, there was no significant difference among priming cohorts in their postvaccination titers to the vaccine viruses. Compared to those that were not infected in 2014–2015, vaccine failure cases had lower cross-reactive antibody titers to circulating viruses and narrower antibody landscapes. However, infection with A(H3N2) viruses in 2014–2015 had little effect on the shape of landscapes following 2015–2016 vaccination. Antibody landscapes offered a comprehensive analysis of an individual's complex antibody profile, allowing the examination of factors that can influence response to vaccination and infection.

In the current study, vaccination with contemporary viruses not only induced antibody rise to vaccine, it also back-boosted antibodies to viruses that children may have been previously exposed to. Yet, in contrast to the "original antigenic sin" hypothesis, the strongest responses detected were against the current vaccine antigens, rather than to the older priming viruses. Of note, in our previous analysis with A(H1N1) landscapes in adults, we detected significant boosts to the likely A(H1N1) priming viruses in addition to strong responses to the vaccine virus following vaccination in 6 influenza seasons [13]. Here, in A(H3N2) antibody landscapes in children, we did not observe a clear boost to the priming virus following vaccination.

When comparing landscapes between seasons, the landscape of each priming cohort was recapitulated in the second season, regardless of the prior season vaccination or infection status, even when there was a significant antigenic drift between the vaccine antigens in the first (Texas/2012 in 2014–2015) and second seasons (Switzerland/2013 in 2015–2016). These data suggest that antibody responses following vaccination were likely derived from expansions of existing memory B cells through the shared epitopes between vaccine and previously exposed viruses. Although higher antibody rise to vaccine viruses may indicate stimulation of naive B cells to the novel epitopes on the new vaccine strain, it likely occurred to a lesser extent given the retained shape of the antibody landscapes. The age effect on antibody landscape was evident: The breadth of the landscapes clearly increased with age, reflecting accumulated exposures over time.

Children without recent influenza vaccination or infection had lower preexisting antibody titers across the landscape than those recently vaccinated or infected, resulting in substantially greater fold-rise after 2015–2016 vaccination, consistent with previous studies [8, 19]. For each birth cohort, despite lower prevaccination titers in the recently unvaccinated and uninfected group, the shape of the landscapes were similar to those among recently vaccinated or infected participants, suggesting similar age-related A(H3N2) priming. Age-related priming, rather than recent vaccination/infection, determined the shape of the antibody landscapes in these children.

It has been hypothesized that binding of vaccine antigens by preexisting antibodies can decrease antigenic load and lead to reduced novel responses following vaccination, termed “antigenic trap” [8]. Although the fold-rise to Switzerland/2013 vaccine was the highest in the recently unvaccinated uninfected cohort, the lack of antigenic trapping potential did not result in higher titers compared to children vaccinated in both seasons; surprisingly, postvaccination landscape titers did not differ significantly between the previously vaccinated and unvaccinated cohorts. Preexisting titers were positively correlated with postvaccination titers for both seasons. This could be interpreted as acquired immunity unhindered by cross-reactive antibodies for “antigenic trapping” [20].

The antigenic distance hypothesis has been cited in explanation for observed low effectiveness in repeat vaccination whereby multiple vaccinations against an identical or antigenically similar component focuses response toward the vaccinating antigen and consequently reduces efficacy against an antigenically drifted epidemiological strain [21, 22]. In our study, repeat vaccination included 2 seasons when an A(H3N2) antigenic cluster transition occurred, from a 3C.1 virus (Texas/2012) in 2014–2015 to a 3C.3a virus (Switzerland/2013) in the following season. The receipt of IIV containing Texas/2012 in 2014–2015 did not predict vaccine response to antigenically distant Switzerland/2013 in the subsequent season. Yet, we observed an incremental increase in titers across the preexisting landscape of the repeat vaccinated cohort (Figure 6B) and demonstrated a positive correlation of postvaccination titer with preexisting titers (Table 1). Of note, in birth cohort 2006–2008 that received LAIV in 2014–2015, there was no antibody landscape increase; here, HI antibodies may not be the best indicator of LAIV vaccine responses. None of our study subjects were infected with A(H3N2) in 2015–2016, this could be an indication of protection conferred by vaccination, or due to low circulation of A(H3N2) (compared to

>80% prevalence of A(H1N1)pdm09) in that season [23]. A larger study is required to adequately address the impact of antigenic distance and antibody landscapes on vaccine effectiveness.

Our study has several limitations. First, the sample sizes of priming cohorts are small, and the narrow age range in the study population (age 7–17 years) may have limited our ability to clearly differentiate the effect of immune priming on vaccine responses, especially for A(H3N2) viruses that have high frequency of antigenic drift. Second, we were not able to use cell-culture propagated viruses to differentiate the effect of egg-adapted substitutions of A(H3N2) viruses in the landscape analysis. Third, our current study is based on HI antibody responses. Additional immunological responses, including neutralizing and neuraminidase inhibition antibodies and other nonneutralizing antibodies, may also contribute to antibody-mediated protection. Furthermore, antibody repertoires deserve further investigation; the persistence of antibody landscape patterns in our study cohorts is consistent with observations of limited clonality of postvaccination repertoire in other studies [24–26]. Last, our findings are focused on A(H3N2); antibody landscape analysis on other influenza subtypes, for example, A(H1N1), also warrants investigation.

An effective vaccination strategy requires stimulation of both de novo antibody responses targeting epitopes specific to the newly emerged, antigenically distinct circulating strains, as well as rapid expansion of existing memory B cells targeting shared epitopes between the circulating strains and viruses from past exposure. It will also need to overcome challenges from the complex, heterogenous preexisting immunity in the human population. Further studies through antibody landscape analysis and other approaches are needed to elucidate immunological determinants of influenza vaccine response and protection, in order to improve influenza vaccine effectiveness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

We thank Dr Feng Liu from the Influenza Division, Centers for Disease Control and Prevention (CDC), for assistance in the study.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agencies.

Financial support.

This study was funded by the CDC, and a cooperative agreement (5 U01IP001038) from the CDC to the Marshfield Clinic Research Institute.

REFERENCES

1. Shim E, Smith KJ, Nowalk MP, et al. Impact of seasonal influenza vaccination in the presence of vaccine interference. Vaccine 2018; 36:853–8. [PubMed: 29329684]
2. Henkle E, Irving SA, Naleway AL, et al. Comparison of laboratory-confirmed influenza and noninfluenza acute respiratory illness in healthcare personnel during the 2010–2011 influenza season. Infect Control Hosp Epidemiol 2014; 35:538–46. [PubMed: 24709723]

3. Mclean HQ, Thompson MG, Sundaram ME, et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. Clin Infect Dis 2014; 59:1375–85. [PubMed: 25270645]
4. Davenport FM, Hennessy AV. A serologic recapitulation of past experiences with influenza A; antibody response to monovalent vaccine. J Exp Med 1956; 104:85–97. [PubMed: 13332182]
5. Davenport FM, Hennessy AV, Francis T Jr. Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. J Exp Med 1953; 98:641–56. [PubMed: 13109114]
6. Lessler J, Riley S, Read JM, et al. Evidence for antigenic seniority in influenza A (H3N2) antibody responses in southern China. PLoS Pathog 2012; 8:e1002802. [PubMed: 22829765]
7. Francis T Jr, Davenport FM, Hennessy AV. A serological recapitulation of human infection with different strains of influenza virus. Trans Assoc Am Physicians 1953; 66:231–9. [PubMed: 13136267]
8. Fonville JM, Wilks SH, James SL, et al. Antibody landscapes after influenza virus infection or vaccination. Science 2014; 346:996–1000. [PubMed: 25414313]
9. Mclean HQ, King JP, Talley P, et al. Effect of previous-season influenza vaccination on serologic response in children during 3 seasons, 2013–2014 through 2015–2016. J Pediat Infect Dis Soc 2020; 9:173–80.
10. Mclean HQ, Caspard H, Griffin MR, et al. Association of prior vaccination with influenza vaccine effectiveness in children receiving live attenuated or inactivated vaccine. JAMA Netw Open 2018; 1:e183742. [PubMed: 30646262]
11. Thompson MG, Naleway A, Fry AM, et al. Effects of repeated annual inactivated influenza vaccination among healthcare personnel on serum hemagglutinin inhibition antibody response to A/Perth/16/2009 (H3N2)-like virus during 2010–11. Vaccine 2016; 34:981–8. [PubMed: 26813801]
12. World Health Organization. Manual for the laboratory diagnosis and virological surveillance of influenza. Geneva, Switzerland: WHO, 2011.
13. Liu F, Tzeng WP, Horner L, et al. Influence of immune priming and egg adaptation in the vaccine on antibody responses to circulating A(H1N1)pdm09 viruses after influenza vaccination in adults. J Infect Dis 2018; 218:1571–81. [PubMed: 29931203]
14. Teros-Jaakkola T, Toivonen L, Schuez-Havupalo L, et al. Influenza virus infections from 0 to 2 years of age: a birth cohort study. J Microbiol Immunol Infect 2019; 52:526–33. [PubMed: 29254653]
15. Silvennoinen H, Huusko T, Vuorinen T, Heikkinen T. Comparative burden of influenza A/H1N1, A/H3N2 and B infections in children treated as outpatients. Pediatr Infect Dis J 2015; 34:1081–5. [PubMed: 26181897]
16. Levine MZ, Martin JM, Gross FL, et al. Neutralizing antibody responses to antigenically drifted influenza A(H3N2) viruses among children and adolescents following 2014–2015 inactivated and live attenuated influenza vaccination. Clin Vaccine Immunol 2016; 23:831–9. [PubMed: 27558294]
17. Skowronski DM, Chambers C, Sabaiduc S, et al. Beyond antigenic match: possible agent-host and immunoepidemiological influences on influenza vaccine effectiveness during the 2015–2016 season in Canada. J Infect Dis 2017; 216:1487–500. [PubMed: 29029166]
18. Flannery B, Smith C, Garten RJ, et al. Influence of birth cohort on effectiveness of 2015–2016 influenza vaccine against medically attended illness due to 2009 pandemic influenza A(H1N1) virus in the United States. J Infect Dis 2018; 218:189–96. [PubMed: 29361005]
19. Luytjes W, Enouf V, Schipper M, et al. HI responses induced by seasonal influenza vaccination are associated with clinical protection and with seroprotection against non-homologous strains. Vaccine 2012; 30:5262–9. [PubMed: 22691431]
20. Fazekas de St G, Webster RG. Disquisitions of original antigenic sin. I. Evidence in man. J Exp Med 1966; 124:331–45. [PubMed: 5922742]
21. Smith DJ, Forrest S, Ackley DH, Perelson AS. Variable efficacy of repeated annual influenza vaccination. Proc Natl Acad Sci U S A 1999; 96:14001–6. [PubMed: 10570188]

22. Skowronski DM, Chambers C, De Serres G, et al. Serial vaccination and the antigenic distance hypothesis: effects on influenza vaccine effectiveness during A(H3N2) epidemics in Canada, 2010–2011 to 2014–2015. J Infect Dis 2017; 215:1059–99. [PubMed: 28180277]

23. Davlin SL, Blanton L, Kniss K, et al. Influenza activity—United States, 2015–16 season and composition of the 2016–17 influenza vaccine. MMWR Morb Mortal Wkly Rep 2016; 65:567–75. [PubMed: 27281364]

24. Lee J, Boutz DR, Chromikova V, et al. Molecular-level analysis of the serum antibody repertoire in young adults before and after seasonal influenza vaccination. Nat Med 2016; 22:1456–64. [PubMed: 27820605]

25. Lee J, Paparoditis P, Horton AP, et al. Persistent antibody clonotypes dominate the serum response to influenza over multiple years and repeated vaccinations. Cell Host Microbe 2019; 25:367–76 e5. [PubMed: 30795981]

26. Wrammert J, Smith K, Miller J, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. Nature 2008; 453:667–71. [PubMed: 18449194]

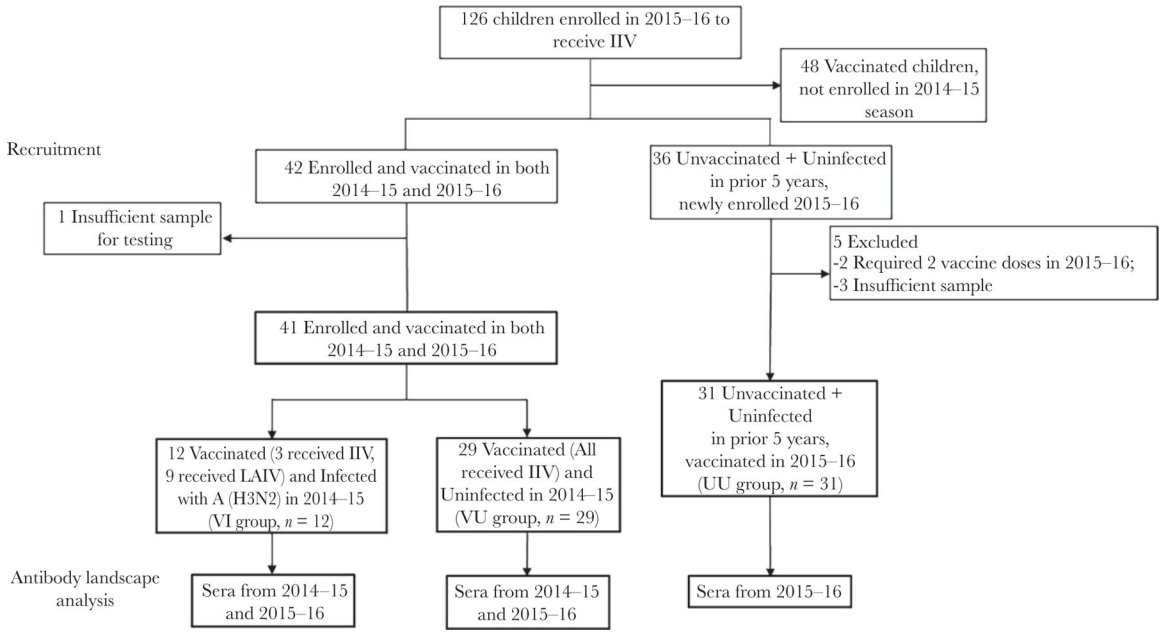

Figure 1.

Study design. Study recruitment and sera used for antibody landscape analysis.

Abbreviations: IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; UU, unvaccinated and uninfected; VI, vaccinated and infected; VU, vaccinated and uninfected.

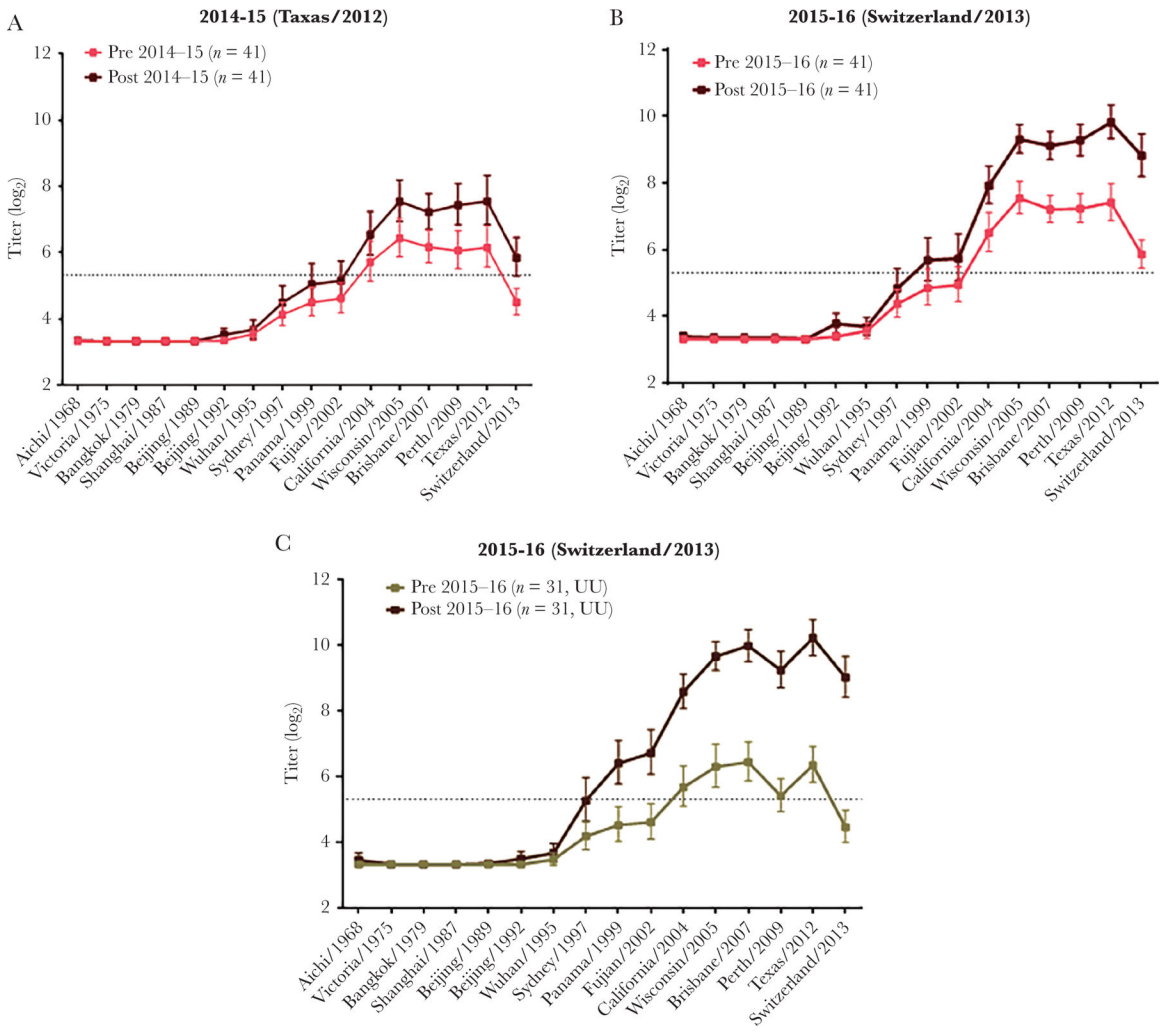

Figure 2.

Antibody landscapes pre- and postvaccination for children vaccinated in both 2014–2015 and 2015–2016 seasons and those only vaccinated in 2015–2016. *A*, Antibody landscapes in 2014–2015 among children vaccinated in both seasons (n = 41). *B*, Antibody landscapes in 2015–2016 among children vaccinated in both seasons (n = 41). *C*, Antibody landscapes in 2015–2016 among children previously unvaccinated and uninfected (UU group, n = 31). Y-axis: $\log_2$ geometric mean titer with 95% confidence interval; dashed line denotes the titer of 40. X-axis: A(H3N2) viruses in chronological order.

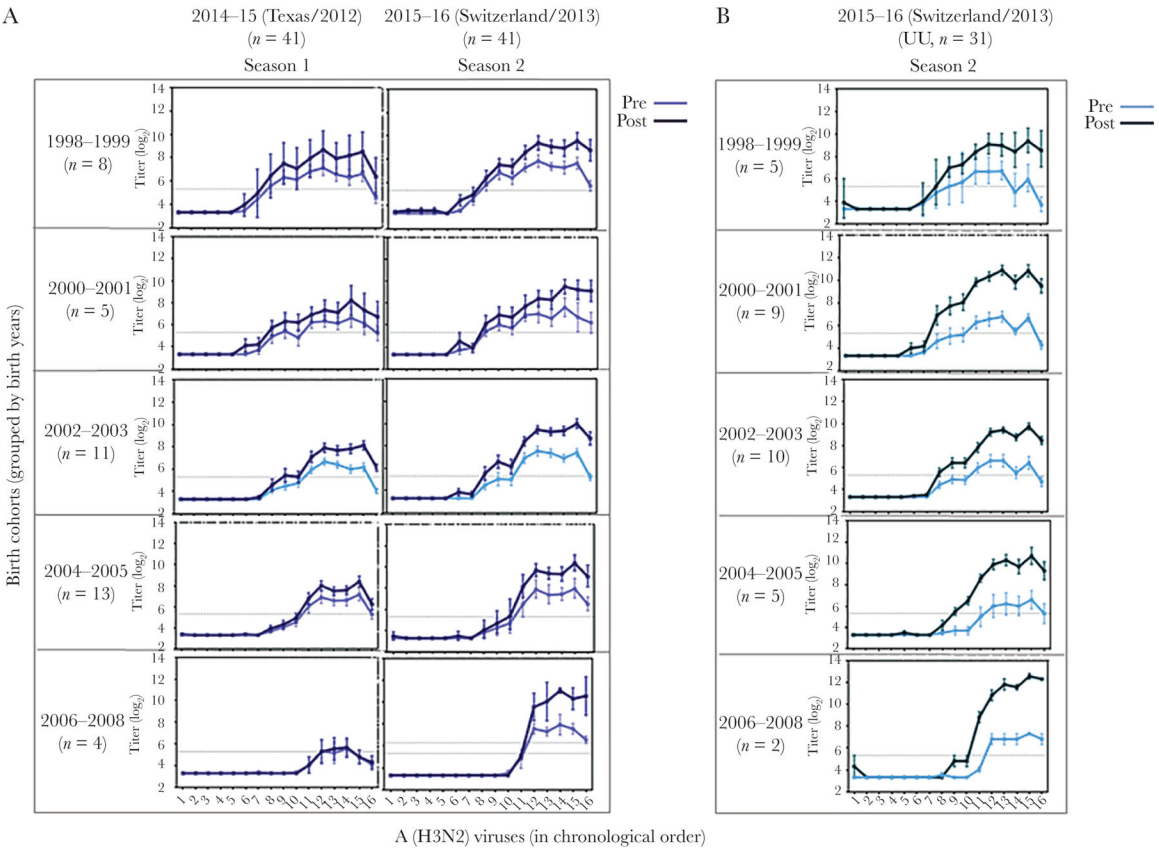

Figure 3.

Antibody landscape changes following vaccination in children grouped by birth cohort. *A*, Antibody landscapes of children vaccinated in both seasons (n = 41) grouped by birth cohort. *B*, Antibody landscapes of children in the unvaccinated and uninfected (UU) group (n = 31) grouped by birth cohort. Y-axis: $\log_2$ geometric mean titer with 95% confidence interval; dashed line denotes the titer of 40. X-axis: A(H3N2) viruses plotted in chronological order by year of isolation: (1) A/Aichi/2/1968, (2) A/Victoria/03/1975, (3) A/Bangkok/1/1979, (4) A/Shanghai/11/1987, (5) A/Beijing/353/1989, (6) A/Beijing/32/1992, (7) A/Wuhan/359/1995, (8) A/Sydney/05/1997, (9) A/Panama/2007/1999, (10) A/Fujian/411/2002, (11) A/California/07/2004, (12) A/Wisconsin/67/2005, (13) A/Brisbane/10/2007, (14) A/Perth/16/2009, (15) A/Texas/50/2012, (16) A/Switzerland/9715293/2013.

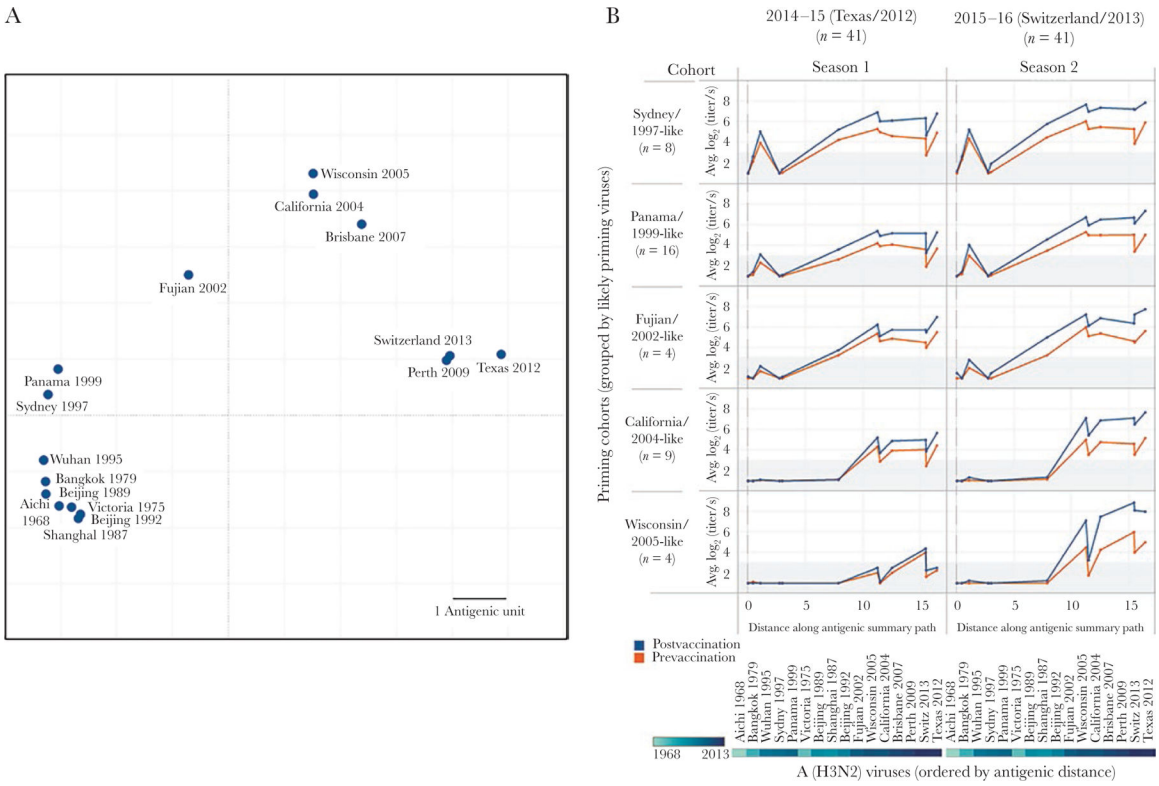

Figure 4.

Antigenic cartographic map of A(H3N2) viruses and antibody landscape changes following vaccination in children grouped by A(H3N2) virus priming cohorts. *A*, Antigenic cartographic map of the 16 A(H3N2) viruses constructed using ferret antisera hemagglutination inhibition titers. Gridlines in the x- and y-axes indicate 1 antigenic unit. *B*, Antibody landscapes for children enrolled in both seasons (n = 41) grouped by 5 immune priming cohorts. Y-axis: average $\log_2$-transformed (titer/5). X-axis: A(H3N2) viruses graphed along the summary path on the x-axis based on antigenic distance of each virus from Texas/2012 calculated from antigenic map in (*A*); see details in the Supplementary Methods.

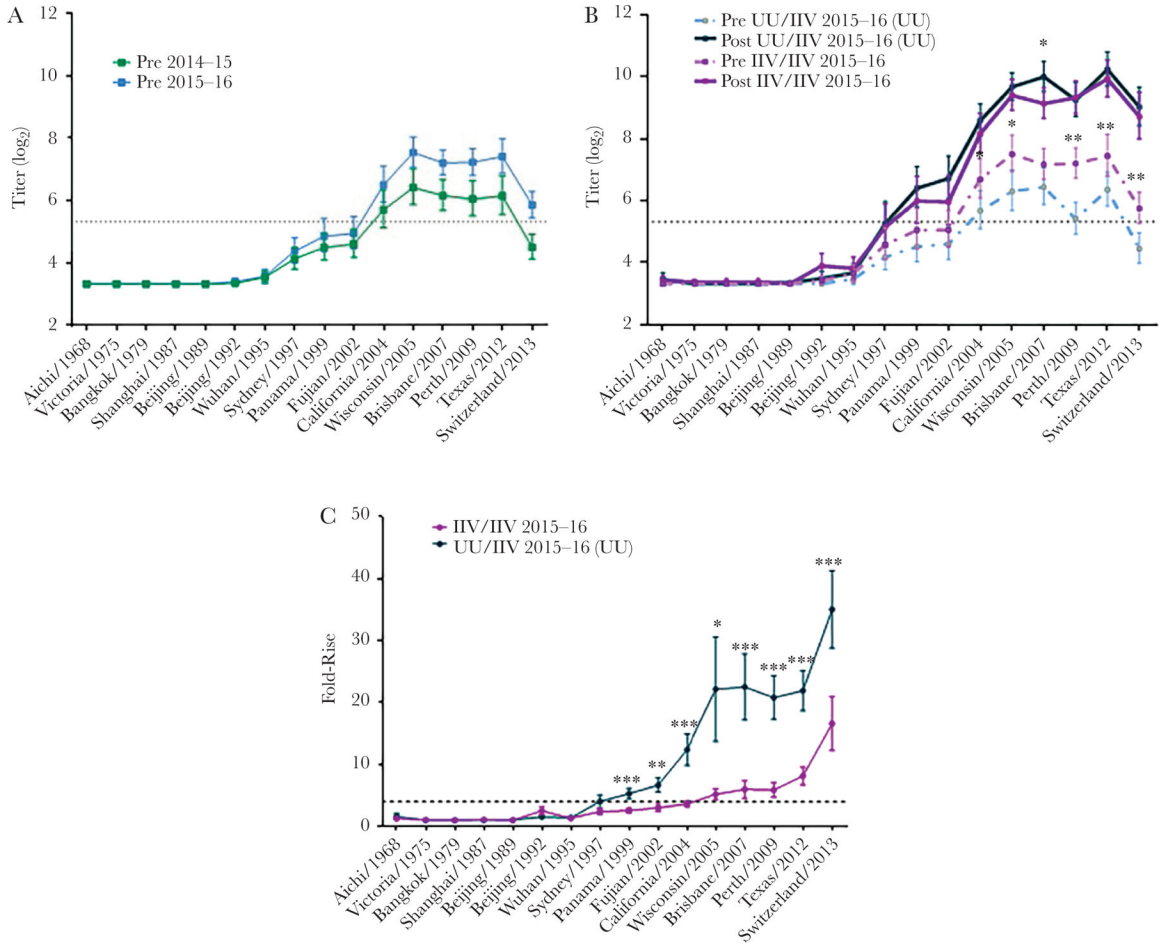

Figure 5.

Impact of prior season vaccination on antibody landscapes changes. *A*, Change of prevaccination antibody landscape in 2014–2015 and 2015–2016 among children enrolled in both seasons (n = 41). *B*, Antibody landscape changes pre- and postvaccination in 2015–2016 stratified by prior season vaccination status. Children who received inactivated influenza vaccine (IIV) in both the 2014–2015 and 2015–2016 seasons (IIV/IIV, n = 32) compared with those in the unvaccinated and uninfected (UU) group who received IIV in 2015–2016 (UU/ IIV, n = 31). Antibody titers to each virus were compared between the 2 groups at pre- and postvaccination. **P* < .05; ***P* < .01. In both (*A*) and (*B*), the dashed line denotes a titer of 40. Y-axis: $\log_2$ geometric mean titer with 95% confidence interval. X-axis: A(H3N2) viruses in chronological order. *C*, Fold-rise to all A(H3N2) viruses following vaccination with Switzerland/2013 in 2015–2016. **P* < .05; ***P* < .01, ****P* < .001; dashed line denotes 4-fold rise. Y-axis: mean fold-rise with standard error. X-axis: A(H3N2) viruses in chronological order. Abbreviations: IIV, inactivated influenza vaccine; LAIV, live attenuated influenza vaccine; UU, unvaccinated and uninfected; VI, vaccinated and infected; VU, vaccinated and uninfected.

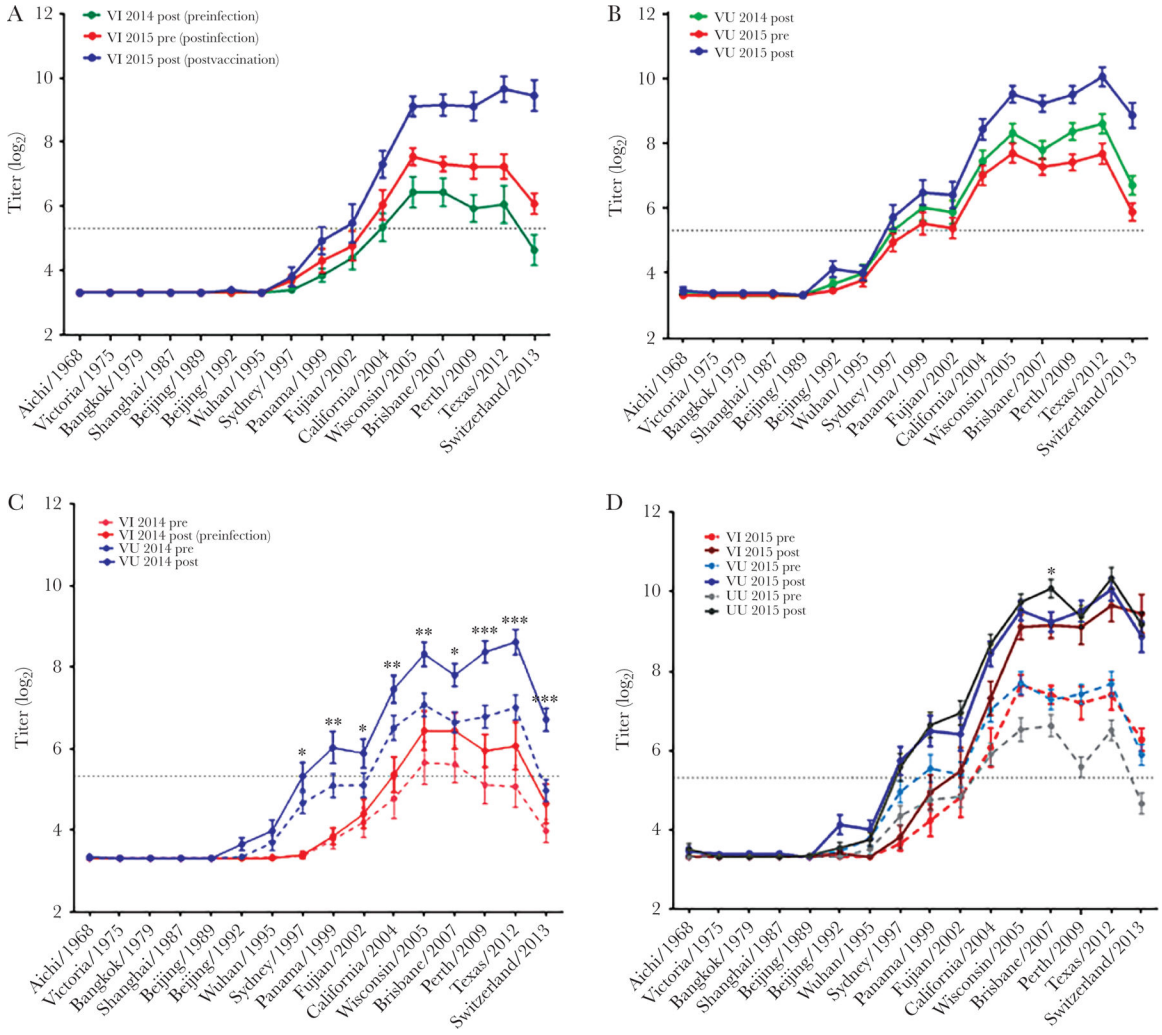

Figure 6.

Impact of prior season infection on antibody landscape changes. *A*, Antibody landscape changes from the 2014–2015 to 2015–2016 seasons in the vaccinated and infected group (VI, n = 12). *B*, Antibody landscape changes from 2014–2015 to 2015–2016 season in the vaccinated but uninfected group (VU, n = 29). *C*, Comparison of pre- and postvaccination antibody landscapes in 2014–2015 among the VI (prior to infection) and VU groups. Statistically significant differences in titers between the VI and VU groups postvaccination for each virus are indicated by $*P < .05$, $**P < .01$, and $***P < .001$. *D*, Comparison of antibody landscapes in 2015–2016 season stratified by prior season (2014–2015) exposure status: VI, VU, and UU (unvaccinated and uninfected, n = 31). Statistically significant difference in titers between the 3 groups postvaccination for each virus were indicated by $*P < .05$. Y-axis: $\log_2$ geometric mean titer with 95% confidence interval. X-axis: A(H3N2) viruses in chronological order. Dashed line denotes a titer of 40.

Table 1.

Predictors of A(H3N2) Antibody Responses Following Inactivated Influenza Vaccination in the 2014–2015 and 2015–2016 Seasons Among Children and Adolescents

Models/Variables	A(H3N2) Response in 2014–2015 to IIV (n = 29)			A(H3N2) Response in 2015–2016 to IIV (n = 72)		
	Parameter Estimate[a]	Standard Error	*P* value	Parameter Estimate	Standard Error	*P* value
Predictors of A(H3N2) antibody responses[a]						
Model 1: Fold-rise in HI titer to 2014–2015 vaccine virus (Texas/2012)						
Age, y	0.016	0.078	0.84			
Prevaccine titer to Texas/2012	−0.476	0.158	0.0122[b]			
Prevaccine titer to likely priming virus[c]	0.379	0.195	0.06			
Model 2: Postvaccination HI titer to 2014–2015 vaccine virus (Texas/2012)						
Age, y	0.016	0.078	0.84			
Prevaccine titer to Texas/2012	0.524	0.158	0.003[b]			
Prevaccine titer to likely priming virus[c]	0.379	0.195	0.06			
Model 3: Fold-rise in HI titer to 2015–2016 vaccine virus (Switzerland/2013)						
Age, y	…	…	…	0.020	0.066	0.77
Prevaccine titer to Switzerland/2013	…	…	…	−0.264	0.162	0.11
Prevaccine titer to likely priming virus[c]	…	…	…	−0.239	0.149	0.11
A(H3N2) infection in 2014–2015	…	…	…	−0.195	0.420	0.64
Receipt of IIV in 2014–2015	…	…	…	−0.557	0.298	0.07
Model 4: Postvaccination in HI titer to 2015–2016 vaccine virus (Switzerland/2013)						
Age, y	…	…	…	0.020	0.066	0.77
Prevaccine titer to Switzerland/2013	…	…	…	0.736	0.162	<0.001[b]
Prevaccine titer to likely priming virus[c]	…	…	…	−0.239	0.149	0.11
A(H3N2) infection in 2014–2015	…	…	…	−0.195	0.420	0.64
Receipt of IIV in 2014–2015	…	…	…	−0.557	0.298	0.07
Association between fold-rise of A/Switzerland and fold-rise of potential priming virus			…			
Model 5: Fold-rise in HI titer to 2015–2016 vaccine virus (Switzerland/2013)			…			

J Infect Dis. Author manuscript; available in PMC 2021 August 03.

Models/Variables	A(H3N2) Response in 2014–2015 to IIV (n = 29)			A(H3N2) Response in 2015–2016 to IIV (n = 72)		
	Parameter Estimate[a]	Standard Error	*P* value	Parameter Estimate	Standard Error	*P* value
Age, y	…	…	…	0.174	0.056	0.003[b]
Prevaccine titer to Switzerland/2013	…	…	…	−0.161	0.118	0.18
Fold-rise in titer to likely priming virus[c]	…	…	…	0.889	0.127	<0.001[b]
A(H3N2) infection in 2014–2015	…	…	…	0.481	0.339	0.16
Receipt of IIV in 2014–2015	…	…	…	−0.308	0.232	0.19

Abbreviations: HI, hemagglutination inhibition; IIV, inactivated influenza vaccine.

[a]Parameter estimates from multivariant linear regression models with log-transformed HI antibody titers.

[b]Statistically significant.

[c]Likely priming virus was defined as the first historic A(H3N2) virus with 2015–2016 postvaccination HI titer ≥40 that circulated after participant's birth year.

J Infect Dis. Author manuscript; available in PMC 2021 August 03.