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An updated influenza A(H3N2) vaccine generates limited antibody responses to previously encountered antigens in children ☆

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ABSTRACT

Background: Influenza vaccination may provide a "back-boost" to antibodies against previously encountered strains. If the back-boost effect is common, this could allow more aggressive vaccine updates, as emerging variants would be expected to both elicit de-novo responses and boost pre-existing responses against recently circulating strains. Here we used the emergence of an antigenically novel A(H3N2) strain to determine whether an antigenically updated vaccine boosted antibodies against historical strains.
Methods: We performed hemagglutination-inhibition (HI) assays on pre- and post-vaccination sera from 124 children 5–17 years old who received 2015–2016 inactivated influenza vaccine, containing an antigenically updated A(H3N2) strain. We evaluated the mean fold increase in HI titer against both the 2015–2016 vaccine strain and representative strains from two prior antigenic clusters. Factors associated with post-vaccination titers against historical strains were evaluated using linear regression, adjusting for baseline titer.
Results: Geometric mean titers against each antigen examined increased significantly after vaccination ($P < .0001$). Mean fold increase was 3.29 against the vaccine strain and 1.22–1.46 against historical strains. Response to vaccine strain was associated with increased post-vaccination titers against historical strains.
Conclusions: A vaccine containing an antigenically novel A(H3N2) strain modestly boosted antibody responses against historical influenza strains in children.

1. Introduction

Influenza viruses undergo continuous antigenic evolution that is punctuated by the periodic emergence of variants with substantial antigenic differences from previously circulating variants. As a result, influenza virus strains can be grouped into clusters of antigenically similar viruses, where new clusters are created during the expansion of a drift variant [1]. These antigenic drift variants can emerge unpredictably and sometimes suddenly, which complicates vaccine strain selection. Decisions regarding the recommended influenza vaccine composition start 6–7 months before the influenza season [2,3]. During this time an antigenically novel strain can emerge, creating a mismatch between vaccine and circulating strains and reducing vaccine effectiveness. Before the 2014–15 influenza season an antigenic cluster transition created a larger-than-usual mismatch between the circulating A(H3N2) strain and the vaccine strain, A/Texas/50/2012. This virus was antigenically similar to a majority of circulating A(H3N2) viruses in early 2014 [4]. However, increasing prevalence of antigenically drifted A(H3N2) viruses during the 2014–15 season resulted in poor A(H3N2)-specific vaccine effectiveness [5,6].

An individual's repertoire of antibody responses is shaped by each influenza exposure event. It has been shown that

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pre-existing immunity as well as vaccine effectiveness can affect the response to vaccination [7,8]. However, there have been few studies examining how an influenza exposure can affect the pre-existing antibody repertoire. Recently, Fonville et al. demonstrated how exposure events alter the overall hemagglutination-inhibition (HI) antibody response by simultaneously visualizing antigenic distances among influenza strains and the magnitude of HI antibody responses to create an "antibody landscape" [9]. Intriguingly, they noted that both infection and vaccination boosted antibody responses against not only the vaccine or infecting strain, but also against historical influenza strains, resulting in a "back-boost" effect [9]. This study also noted that vaccination with an updated influenza vaccine in 1997 was capable of boosting responses against the previously circulating antigenic variant A/Wuhan/359/1995 to levels higher than those elicited by the homologous A/Nanchang/933/1995 vaccine [9].

The "back-boost" effect raises the possibility of employing a new vaccine strain selection process in which vaccines can be updated with emerging, antigenically novel strains, even when they do not yet predominate among circulating viruses. The antigenically advanced strain would be relied upon to elicit antibodies against the emerging antigenic variant while also boosting antibodies against historical strains, thus providing coverage against both "new" and "old" strains. The back-boost effect was initially identified in adults and relies on pre-existing influenza immunity; here we determined whether a similar back-boost could occur in children who have comparatively limited influenza exposure histories. The study recruited children with and without a known prior infection with influenza A(H3N2) viruses, which allowed us to examine factors associated with a back-boost response.

2. Methods

2.1. Study population and serum collection

This study was carried out in fall 2015 before the North American 2015–2016 influenza season. Parents of children 5–17 years old were contacted by mail and telephone inviting their child to participate in a vaccine immune response study before they received their 2015–2016 influenza vaccine. Participants were selected from children who (1) previously participated in similar influenza vaccine immune response studies in the previous two influenza seasons, or (2) lived in Marshfield, Wisconsin where annual studies of influenza vaccine effectiveness were conducted [10]. We restricted recruitment to this population because influenza vaccinations are captured using a validated vaccination registry [11] and medically attended influenza illnesses were prospectively identified and confirmed by RT-PCR. All participants received the 2015–2016 trivalent inactivated influenza vaccine (IIV3) delivered intramuscularly, containing an A/Switzerland/9715293/2013-like A(H3N2) virus, and provided a serum sample before vaccination and 28 days after vaccination. Data on demographics and high-risk conditions were electronically extracted from the medical record.

Study procedures were approved by the Marshfield Clinic Institutional Review Board. Informed consent was obtained from the parents/guardians of all participants and assent was obtained from children aged $\geq$7 years.

2.2. Hemagglutination inhibition (HI) assay

HI assays were performed at the University of Wisconsin-Madison on pre-baseline and post-vaccination serum samples as described by the World Health Organization [12]. Briefly, 1 part serum was mixed with 3 parts receptor-destroying enzyme II (RDEII; Accurate Chemical, Westbury, NY) and incubated at 37 °C for 18–20 h to remove non-specific inhibitors of hemagglutination. RDEII was then inactivated by incubating the samples at 56 °C for 30 min. To remove non-specific antibodies 1 part packed guinea pig red blood cells (Innovative Research, Novi, MI) was mixed with 10 parts serum and incubated at room temperature for 1 h, inverting every 15 min. The samples were then diluted to a total serum dilution factor of 1:10 with PBS and oseltamivir (final concentration 20 nM; ApexBio, Houston, TX) and serial twofold dilutions were mixed with 4 HA units of A(H3N2) viruses in a U-bottomed microtiter plate. The plates were incubated at room temperature for 30 min, then 0.75% guinea pig red blood cells were added with a final oseltamivir concentration of 20 nM. The samples were then incubated again for 30 min at room temperature. The reciprocal of the dilution at which no inhibition was observed was recorded as the HI antibody titer. If inhibition was not observed a serum dilution of 1:5 was recorded as a negative result to allow for statistical analysis. Samples were tested in duplicate with pre- and post-vaccination samples from the same participants tested at the same time. Wells with 4 HA units of virus and PBS were kept as positive and negative controls for hemagglutination.

Antibody titers were determined against the following viruses: the 2015–2016 vaccine strain A/Switzerland/9715293/2013 (SW/13) (ATCC, Manassas, VA) belonging to the genetic clade 3C.3a; the 2014–2015 vaccine strain A/Texas/50/2012 (TX/12) (ATCC, Manassas, VA) belonging to clade 3C.1; the 2012–2013 vaccine strain A/Victoria/361/2011 (VI/11) (ATCC, Manassas, VA) belonging to clade 3C.1; and A/Wuhan/359/1995 (WU/95) (kindly provided by Dr. Yoshihiro Kawaoka) which represented an A (H3N2) antigenic cluster that circulated from 1995 to 1997, before almost all the participants were born [13]. Viruses were grown in cell culture using MDCK-SIAT1 cells.

2.3. Statistical analyses

The primary outcome was geometric mean fold increase (MFI) in HI titers against TX/12; MFI was also assessed for VI/11, a virus from the same antigenic cluster as TX/12, and for WU/95, which is antigenically distinct from TX/12. An analysis of MFI was stratified based on baseline TX/12 HI titer <40 or $\geq$ 40 to account for potential differential antibody response due to baseline TX/12 HI titer. Seroprotection against the previous season vaccine strain, TX/12, was determined for two levels of protection, defined as the proportion with post vaccination HI titer $\geq$40, the titer level considered seroprotective for regulatory purposes; and $\geq$110, which has been suggested to correspond to 50% clinical protection in children [14,15].

Linear regression models with $\log_2$-transformed HI titers were used to identify factors associated with post-vaccination HI titer against viruses representing past A(H3N2) antigenic clusters, adjusting for baseline HI titers. Factors examined included SW/13 (vaccine strain) MFI, age group (5–11 and 12–17 years), presence of a high-risk condition, vaccination group (any vs. no vaccination within the last 5 years), and A(H3N2) infection history within the last 5 years. High-risk conditions were defined by presence of a healthcare visit or hospitalization during the previous 12 months with $\geq$ 1 ICD-9 diagnosis codes for conditions associated with increased risk of influenza, as previously described [16,17]. Covariates associated with post-vaccination titer at $P < .05$ in backwards elimination were included in the final linear regression model. Separate regression models were used to assess titers against each historical strain: TX/12, VI/11, and WU/95. Estimates of post-vaccination geometric mean titers (GMT) for each historical antigen were calculated and plotted adjusting for the baseline GMT for each antigen using "predicted marginal means" or "least-squares means," available in the R package lsmeans [18].

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