Estimation of the Association Between Antibody Titers and Protection Against Confirmed Influenza Virus Infection in Children

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Antibody titers measured by hemagglutination inhibition (HAI) correlate with protection against influenza virus infection and are used to specify criteria for vaccine licensure. In a randomized, controlled trial of seasonal influenza vaccine in 773 children aged 6–17 years, we estimated that HAI titers of 1:40 against A(H1N1)pdm09 and B(Victoria lineage) were associated with 48% (95% confidence interval [CI], 30%–62%) and 55% (95% CI, 32%–70%) protection against PCR-confirmed infection with each strain. Our analysis accounted for waning in antibody titers over time, and could be particularly useful in settings where influenza activity is delayed or prolonged relative to measurement of antibody titers.

Keywords. antibody; immunity; influenza; vaccine; children.

BACKGROUND

Higher titers of hemagglutination-inhibiting (HAI) antibody are correlated with protection against influenza virus infection [1, 2]. A classic study compared risks of infection in adult volunteers with various HAI antibody titers prior to artificial challenge [1], forming the basis for requiring influenza vaccines to induce an HAI antibody titer of ≥1:40 in order to ensure at least 50% protection against infection [3]. However, the degree of protection associated with specific HAI titers is based on limited data, particularly for children [2–6].

Our aim was to estimate the relationship between postvaccination antibody titer and risk of influenza virus infection, accounting for waning in antibody titers over time in the absence of infection and allowing risk of infection to vary over time. By applying this approach to data from a large, randomized, placebo-controlled trial of trivalent inactivated influenza vaccine (TIV) efficacy in children in Hong Kong [7], we provide estimates of the correlation between HAI titer and protection in children aged 6–17 years.

MATERIALS AND METHODS

Participants and Follow-up

The TIV contained 15 μg of hemagglutinin for each of the A/Brisbane/59/2007(H1N1)–like, A/Brisbane/10/2007(H3N2)–like, and B/Brisbane/60/2008–like (Victoria lineage) strains. Participants and their household contacts were followed for 9–12 months for signs and symptoms of acute respiratory illness (ARI; any 2 of the following: tympanic temperature ≥37.8°C, cough, sore throat, headache, chills, coryza, and myalgia) by daily symptom diaries and biweekly telephone interviews. They were advised to call the study hotline as soon as possible after onset of an ARI in any household member. Home visits were arranged immediately to households in which any members reported ARI. During each visit by a study nurse, nose and throat swabs (NTS) were collected from each household member, regardless of presence or absence of illness, for testing by reverse transcriptase-polymerase chain reaction (PCR). NTSs were eluted and cryopreserved at −70°C after receipt until testing at the end of the study. The PCR assay targeted the matrix gene; influenza B lineage differentiation was done by lineage-specific PCR assay targeting the HA gene.

Serum specimens were collected immediately from all participants before (prevaccination) and 1 month after receipt of TIV/placebo (postvaccination) and at the end of follow-up in August–December 2010 (end-of-study) and from 25% of participants in April–May 2010 (midstudy). The sera were tested in parallel by HAI assays against the vaccine strain B/Brisbane/60/2008–like (Victoria lineage), the 2009 pandemic strain A/California/7/2009(H1N1pdm09), and another circulating strain B/Florida/4/2006 (Yamagata lineage). Sera were tested in serial doubling dilutions from an initial dilution of 1:10 to endpoint by HAI using standard methods [7, 8].
Proxy written consent was obtained from the parents or legal guardians of the children, with additional written consent from children aged 8–17 years. The Institutional Review Board of Hong Kong University approved the study.

Statistical Methods
We first estimated the rate of waning in HAI titers against each virus strain in the absence of infection using log-linear regression models based on all postvaccination, mid-study, and end-of-study antibody titer data, separately analyzing participants who received TIV or placebo and excluding participants who may have been infected with influenza during follow-up. The models also allow for the possibility of different antibody waning rates between children aged 6–8 years and 9–17 years. To avoid inclusion of infections in these analyses and consequently biasing the estimates of waning rates, we excluded participants with the top 20% (H1N1pdm09) and 15% (B(Victoria lineage)) of HAI titer changes during the follow-up period based on the proportions of participants in the placebo group with serologic evidence of infection (see Supplementary Appendix). Sensitivity analyses using alternative assumptions were performed by assuming a higher percentage of children infected and by assuming infection only occurred in the older children with PCR-confirmed infection and including all other children in the analyses (see Supplementary Appendix).

Having established the waning rates for each virus strain, we used these rates to extrapolate the daily HAI titers for each participant (in the absence of infection) based on their postvaccination HAI titer, age, and receipt of TIV or placebo. We then used Cox proportional hazard models to estimate the correlation of HAI titer over time with protection against PCR-confirmed infection and to investigate whether this association differed for participants who received TIV or placebo, allowing for time-varying influenza incidence in the community (see Supplementary Appendix). The reduction in risk of PCR-confirmed influenza at each HAI titer was estimated via (1 minus hazard ratio) relative to the risk at an HAI titer <1:10. All statistical analyses were conducted using R version 2.15.2. Data and R syntax to reproduce these analyses are available from the corresponding author.

RESULTS
Of 796 randomized participants, postvaccination HAI titers were not available for 23, and these participants were excluded from all analyses. The remaining 773 participants included 465 allocated to TIV and 308 to placebo. The participants who received TIV and placebo shared similar characteristics (Supplementary Appendix Table 1). Figure 1 illustrates the periods of vaccination and serum collection in relation to influenza detections in the study participants, as well as community-based influenza surveillance data. Before vaccination, only 23% of participants had HAI antibody titer ≥1:10 against B/Brisbane/60/2008–like virus. These participants were more likely to have postvaccination HAI titer of ≥1:40 compared with participants who had lower prevaccination HAI titers (49% vs 21%; P value <.01). During the study period there were epidemics of influenza A(H1N1)pdm09 and B(Victoria lineage); Figure 1. During the follow-up period, 1446 swabs were collected from 352 children, and 31 children were positive by PCR for influenza B(Victoria lineage), 15 for A(H1N1)pdm09, 8 for A(H3N2), and 4 for influenza B(Yamagata lineage) viruses (Figure 1). Because of the small number of PCR-confirmed A(H3N2) and B(Yamagata lineage) infections, our analyses focused on B(Victoria lineage) and A(H1N1)pdm09. While A(H1N1)pdm09 was not included in the TIV, the circulating B(Victoria lineage) virus remained antigenically close to the vaccine strain based on phylogenetic analysis of the HA gene [9].

For A(H1N1)pdm09 there was no evidence that the antibody waning rate differed by age and similarly for B(Victoria lineage) among the placebo recipients. However, for B(Victoria lineage) antibody titers among the TIV recipients, antibody titers declined more quickly in younger (6–8 years) compared with older (9–17 years) participants (Supplementary Appendix Table 2). We estimated that 2-fold declines in HAI titers against A(H1N1)pdm09 occurred after means of 187 (95% CI, 150–248) and 205 (95% CI, 159–289) days for children who received TIV and placebo, respectively (Figure 1, Supplementary Appendix Table 2). For B(Victoria lineage), 2-fold declines in HAI titers occurred after means of 96 (95% CI, 83–113) and 132 (95% CI, 115–156) days (P <.01) for the children aged 6–8 years and 9–17 years, respectively, who received TIV. For placebo recipients, the 2-fold decline in B(Victoria lineage) HAI titers occurred after a mean of 224 (95% CI, 150–437) days. In sensitivity analyses, HAI titers declined at similar rates as in the main analyses if a higher percentage of children with the highest geometric change in HAI titer (assumed as potentially infected during the study period) were included. The waning rate was estimated to be slower if only children with PCR-confirmed infection were excluded (Supplementary Appendix Figure 1 and Supplementary Appendix Table 2).

Accounting for waning in antibody titers and for the time-varying community risk of infection, higher HAI titers corresponded to a greater reduction in risk of PCR-confirmed infection, and the correlation was independent of vaccination status for both A(H1N1)pdm09 and B(Victoria lineage). An HAI titer of 1:40 against A(H1N1)pdm09 correlated with 48% (95% CI, 30%–62%) protection against PCR-confirmed infection with A(H1N1)pdm09, while an HAI titer of 1:40 against B(Victoria lineage) corresponded to 55% (95% CI, 32%–70%) protection against PCR-confirmed infection with B(Victoria lineage; Figure 1). Figure 2 shows the estimated protection against B (Victoria lineage) at 1 to 9 months following receipt of TIV based on the projected HAI titer in individual participants.
Receipt of TIV was associated with a median of 75% protection at 1 month in children aged 9–17 years. The estimated protection declined slowly over time; however, >50% protection remained for most children at 9 months. The estimated level of protection at 1 month was lower in children aged 6–8 years (median protection 61%) and declined more quickly.

In a sensitivity analysis without correcting for antibody waning, the titers required for 50% protection were slightly lower than in the main analysis (Supplementary Appendix Figure 1). In a separate sensitivity analysis, the estimated 50% protective HAI titer was similar when HAI titers against influenza B(Victoria lineage) were correlated with protection.

Figure 1. A, Timeline of the study. Dashed line showing cumulative percentage of 773 participants who received TIV/placebo, provided postvaccination serum specimens, and provided poststudy sera at the end of follow-up; shaded area indicates the follow-up period. In addition, midstudy sera were collected from 25% of participants in April–May 2010. The overlaid histogram displays the monthly numbers of polymerase chain reaction (PCR)-confirmed influenza virus infections in the study participants. B, Influenza activity in the community was based on a proxy measure calculated as the product of the weekly proportion of influenza-like illness consultations among outpatient visits reported by the local Centre for Health Protection multiplied by the weekly proportion of detections of each influenza strain among specimens tested by the local Public Health Laboratory Service. C and D, Postvaccination, midstudy, and poststudy hemagglutination-inhibiting (HAI) antibody titers against influenza A(H1N1)pdm09 and B(Victoria lineage) virus in children who received trivalent inactivated influenza vaccine (TIV, represented by red circles) or placebo (represented by gray circles). Of the 773 participants, 770, 281, and 729 provided prevaccination, midstudy, and end-of-study serum, respectively. Regression lines for each group (TIV, red; placebo, gray), accounting for repeated measures are also shown to indicate the rates of antibody waning. E and F, Correlation between HAI titers and protection against PCR-confirmed influenza A(H1N1)pdm09 and B(Victoria lineage) virus infection. Antibody titers were measured by HAI using A/California/04/2009 and B/Brisbane/60/2008–like (Victoria lineage) viruses, respectively. Protection was calculated as the relative risk reduction compared with the risk at an HAI titer <1:10, corrected for waning in HAI titers throughout the follow-up period in the absence of infection.
against all PCR-confirmed influenza B infections (predominantly by Victoria lineage viruses). An HAI titer of 1:40 against B (Yamagata lineage) was correlated with substantially lower protection against PCR-confirmed influenza B infection (predominantly by Victoria lineage viruses; data not shown).

DISCUSSION

One criterion for influenza vaccine licensure is that at least 70% of vaccine recipients aged 18–60 years and 60% of recipients aged >60 years achieve a postvaccination HAI titer $\geq 1:40$ [3]. This recommendation is based on evidence that an HAI titer of 1:40 correlates with approximately 50% clinical protection [1, 2]. Our findings are consistent with the threshold applying for children aged 6–17 years both for a B(Victoria lineage) virus, which was included in the vaccine, and A(H1N1)pdm09, which was not included in the vaccine (Figure 2). Our results should contribute to the ongoing discussion on whether vaccine licensure requirements should be amended to ensure better protection in children [3]. In our analysis, inclusion of time-varying risk of infection improved the model fit, and correcting for antibody waning avoided underestimation of the protection associated with particular HAI titers (Supplemental Appendix Figure 1).

Early data from experimental challenge studies was commonly used to estimate the 50% protective HAI titer [1, 2]; in those studies it is not necessary to account for waning because antibody titers can be measured at the time of challenge. Our approach corrects for variability over time in antibody titers, which peak at around 2–4 weeks after vaccination and then gradually wane over time [10, 11]. While volunteer challenge studies or community-based studies can be used to determine the correlation between HAI titers and protection in adults, community-based studies provide the best evidence on this correlation in children. Our approach would be particularly useful in settings with delayed or prolonged periods of influenza activity. Our estimates of the rate of waning in HAI titers in children who received TIV were similar to those in another study, where the mean time to 2-fold decline in HAI titer in the absence of infection was 111 days [6]. In addition, we estimated faster waning of HAI titers against influenza B(Victoria lineage) in TIV recipients (Figure 2).

We did not find any evidence for a difference in the protection against infection associated with an HAI titer of 1:40 in the TIV vs placebo group. The preexisting A(H1N1)pdm09 antibodies detected in our participants were probably derived from natural infection during the 2009 pandemic because preexisting antibody against A(H1N1)pdm09 was low before 2009 and none of the participants reported receipt of the A(H1N1)pdm09 vaccine [12]. Because antibody against influenza B(Victoria lineage) was elevated substantially by vaccination [7], our results are consistent with a similar level of protection associated with HAI titers whether from natural infection or boosted by vaccination. In addition, HAI antibody against B(Yamagata lineage) was found to correlate with substantially lower protection against Victoria-lineage viruses. This implies that including both Victoria- and Yamagata-lineage viruses in influenza vaccines could provide more complete protection when viruses of both lineages are circulating.

There are some limitations of our study. First, while we confirmed 58 infections in study participants by PCR, evidence from postseason serology suggested a 3-fold higher cumulative incidence of infection, and it is likely that we did not confirm all infections [7, 12]. However, serology may be less sensitive in

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**Figure 2.** The estimated protection against infection among study participants, based on hemagglutination-inhibiting (HAI) antibody titers at 1 to 9 months following receipt of trivalent inactivated influenza vaccine. The estimated protection for participants at the 25th, 50th, 75th, and 100th percentile of HAI titer are shown for (A) participants aged 6–8 years and (B) participants aged 9–17 years.
detecting infection in children with higher postvaccination antibody titers [13], and the optimal use of serologic data to ascertain infection rates has not yet been clarified [14, 15].

There are likely to be other correlates of protection apart from HAI titers, and our methods could be applied to other data in the future, for example, on antibody titers measured by virus neutralization. Finally, the use of surveillance data as a proxy measure of risk of infection may not fully represent the risk of infection over time for specific individuals, and additional information on risk of infection could be incorporated into the model if available.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Calvin Cheng, Daniel Chu, Winnie Lim, Edward Ma, Hau Chi So, and Jessica Wong for research support. We thank Susan Chiu and Alex Cook for helpful discussion.

Financial support. This project was supported by the Harvard Center for Communicable Disease Dynamics from the National Institute of General Medical Sciences (grant U54 GM088558), the Research Fund for the Control of Infectious Disease, Food and Health Bureau, Government of the Hong Kong SAR (grants CHP-CE-03 and PHE-2), and the Area of Excellence Scheme of the Hong Kong University Grants Committee (grant AoE/M-12/06). The funding bodies had no role in study design, data collection, and analysis, preparation of the manuscript, or the decision to publish.

Potential conflicts of interest. D. K. M. I. has received research funding from F. Hoffman-La Roche Ltd, J. S. M. P. receives research funding from Crucell NV and serves as an ad hoc consultant for GlaxoSmithKline and Sanofi. B. J. C. has received research funding from MedImmune Inc., and consults for Crucell NV. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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