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Original Article

Absence of cross-reactive antibodies to influenza A (H1N1) 2009 before and after vaccination with 2009 Southern Hemisphere seasonal trivalent influenza vaccine in children aged 6 months–9 years: a prospective study

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Background Early outbreaks of the pandemic influenza A (H1N1) 2009 virus predominantly involved young children, who fuelled transmission through spread in homes and schools. Seroprevalence studies conducted on stored serum collections indicated low levels of antibody to the novel strain in this age group, leading many to recommend priority immunisation of paediatric populations.

Objectives In a prospective study, we sought evidence of cross-reactive antibodies to the pandemic virus in children who were naïve to seasonal influenza vaccines, at baseline and following two doses of the 2009 Southern Hemisphere trivalent influenza vaccine (TIV).

Patients/Methods Twenty children were recruited, with a median age of 4 years (interquartile range 3–5 years); all received two age appropriate doses of TIV. Paired sera were collected pre- and post-vaccination for the assessment of vaccine immunogenicity, using haemagglutination inhibition and microneutralisation assays against vaccine-related viruses and influenza A (H1N1) 2009.

Results Robust responses to H3N2 were observed regardless of age or pre-vaccination titre, with 100% seroconversion. Fewer seroconverted to the seasonal H1N1 component. Only two children were weakly seropositive (HI titre 40) to the pandemic H1N1 strain at study entry, and none showed evidence of seroconversion by HI assay following TIV administration.

Conclusions Administration of 2009 Southern Hemisphere TIV did little to elicit cross-reactive antibodies to the pandemic H1N1 virus in children, in keeping with assay results on stored sera from studies of previous seasonal vaccines. Our findings support the recommendations for influenza A (H1N1) 2009 vaccination of children in preparation for the 2010 winter season.

Keywords Australia, H1N1 subtype, human, influenza, influenza A virus, influenza vaccines, pandemic, paediatrics.

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Background

The World Health Organisation declared an influenza pandemic in June 2009, following global spread of a novel reassortant swine-origin influenza strain that emerged in the Americas [influenza A (H1N1) 2009].[1] Early epidemiologic reports indicated higher symptomatic attack rates in children than adults, with variable experience of disease severity.[2–4] Established transmission of the new strain within Australia, one of the earliest countries in which the infection was introduced during the Southern Hemisphere winter, was first described among school children in the Northern suburbs of Melbourne.[5] While close mixing in institutional environments such as schools undoubtedly facilitated spread of the virus,[6] detailed study of outbreaks revealed that children were both more infectious[5] and more

susceptible[7] than adults with whom they were in close contact.

Seroprevalence studies conducted on stored sera from clinical vaccine trials conducted in the United States (US) demonstrated a positive correlation between age and cross-reactive antibody to the novel strain.[8,9] Immunity in the elderly, inferred from a lower clinical attack rate, has since been attributed to exposure to antigenically related H1N1 viruses circulating in the early decades of the 20th century.[10] The absence of such protection in children drove recommendations for priority immunisation of the paediatric age group with strain-specific H1N1 vaccines both to provide direct protection and to reduce community-level transmission.[11] This study was initiated in the first weeks of the Australian pH1N1 outbreak to provide local data to inform immunisation policy, including the potential use of seasonal vaccines for partial protection should this be observed. These data further add to an emerging body of knowledge describing baseline characteristics and subsequent disease experience of disparate populations.

Objectives

This prospective, open-label clinical trial sought to recruit 40 influenza vaccine-naïve children between 6 months and 10 years of age in suburban Melbourne during the 2009 Southern Hemisphere influenza season, prior to availability of strain-specific pandemic vaccines. Its purpose was to measure existing cross-reactive antibody against influenza A (H1N1) 2009 (A/California/4/2009-like) (pH1N1) viruses at baseline and seek evidence of induction of such responses to the novel strain following the receipt of two doses of the 2009 seasonal trivalent influenza vaccine (TIV), which contained antigens from a different H1N1 strain (A/Brisbane/59/2007).

Patients/methods

Study population

We aimed at recruiting 20 healthy children in each of two age strata: Cohort A, aged ≥6 months to <3 years; Cohort B, aged ≥3 years to <10 years. To be eligible, participants were required to be born at full term and in good health. Exclusion criteria were receipt of any prior influenza vaccine; hypersensitivity to any vaccine component, including eggs; confirmed or suspected immune deficiency or recent immunosuppressive therapy; recent receipt of immunoglobulins or blood products; anticoagulant therapy; recent or planned receipt of an investigational compound or any other clinical indication that the investigator deemed sufficient to preclude study participation. Evidence of significant active infection and/or fever necessitated deferral of study entry.

Study procedures

Eligible subjects received two doses of 2009 Southern Hemisphere formulation seasonal inactivated TIV manufactured (and provided free of charge) by CSL Ltd, Parkville, Victoria, Australia, administered 30 (+5) days apart. Participants in Cohort A received two 0·25 -ml vaccine doses, while those in Cohort B received two 0·5 -ml vaccine doses, consistent with national guidelines.[12] Blood samples to assess vaccine immunogenicity were collected at baseline and 30(+5) days after the second vaccine dose.

Assessment of antibody titres to influenza strains

Antibody responses to seasonal vaccine and influenza A (H1N1) 2009 influenza strains were measured in paired sera collected before and after completed vaccination. Responses to influenza virus antigens were measured by haemagglutination inhibition (HI)[13] and virus microneutralisation (MN)[14] assays at the World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne (WHO CC).

HI assays were performed as previously described.[13] Viruses were passaged in embryonated hen's eggs and stored at −80°C. A/California/7/2009 virus was further purified by sucrose gradient, concentrated and inactivated with β-propiolactone, to create an influenza zonal pool (IZP) preparation (provided by CSL Ltd). All serum samples were assayed against IZP-A/California/7/2009 virus (pH1N1) and whole, live A/Brisbane/59/2007 (H1N1) and A/Brisbane/10/2007 (H3N2) viruses. Titres were expressed as the reciprocal of the highest dilution of serum where haemagglutination was prevented.

For MN assays, serum samples were assayed against egg-grown A/Auckland/1/2009 (A/California/7/2009-like) (pH1N1), A/Fukushima/141/2006 (A/Solomon Islands/3/2006-like) (H1N1) and A/Brisbane/10/2007 (H3N2) viruses. Serum was inactivated at 56°C for 30 min, then two-fold dilutions of serum (from 1:10 to 1:1280) were mixed with 200 50% tissue culture infectious dose ($TCID_{50}$) of each virus (1:1, v/v) and the samples incubated at 35°C for 1 h. Serum/virus mixes were added to washed MDCK monolayers in 96-well flat-bottomed plates and incubated at 35°C, 5% CO_2 for 1 h. Samples were replaced with serum-free medium containing 4 μg/ml trypsin and plates incubated for a further 4 days. Virus was detected in the supernatant by the addition of 25 μl 1% turkey RBC. Wells containing fully haemagglutinated RBC were scored positive. Titres were expressed as the reciprocal of the highest dilution of serum where haemagglutination was prevented. Samples were analysed in duplicate in both HI and MN assays. Duplicate titres differed by no more than twofold. Where there was a twofold discrepancy between titres, the lower value was used in analyses.

Immunogenicity was assessed according to the criteria for the evaluation of interpandemic influenza vaccines using HI assays in adults aged 18–64 years developed by the Committee for Proprietary Medicinal Products (CPMP/BWP/214/96) and included a measure of the central tendency of the range of titre distributions, seropositive proportion (titre of ≥40) and seroconversion rate defined as at least a fourfold rise in paired titres.

Ethical approvals

Ethical approval for this study was received from the Royal Children's Hospital Human Research Ethics Committee (HREC Approval # 29055). Written informed consent was obtained from the parent or guardian of study participants prior to study enrolment. All study procedures were performed in accordance with International Conference on Harmonization Good Clinical Practice guidelines.

Results

Study population

Despite study commencement in May 2009, this protocol overlapped the recruitment phase for an urgent pandemic H1N1 vaccine trial,[15] making us ethically obliged to offer parents the choice of participation in one of two influenza vaccine studies. In consequence, only 20 children were enrolled – 3 in Cohort A and 17 in Cohort B. The median age of participants was 4 years (IQR 3–5 years, range 7 months to 7 years). Given the smaller than anticipated sample size and limited distribution of participant ages, results were pooled for analysis. Disposition of study participants and samples throughout the trial is outlined in Figure 1.

Responses to seasonal vaccine antigens

Half of the study participants demonstrated antibodies (HI titre ≥40) to H3N2 at baseline, and 100% of those with post-vaccination sera were seropositive by the end of the study. Robust immune responses to the H3 component were observed regardless of baseline serostatus or age, with 100% seroconversion (Table 1). Given the asymmetric distribution of responses, which did not approximate a normal distribution after log transformation, medians, rather than arithmetic or geometric means, are reported as measures of central tendency. Fewer children were seropositive to the seasonal H1N1 strain than to H3N2 on enrolment (20% by HI or MN assay), and seroconversion rates to this antigen were lower (Table 1).

Cross-reactivity to influenza A (H1N1) 2009

Only two children had any evidence of cross-reactive antibody to pH1N1 at study entry, with MN titres of 40. Both

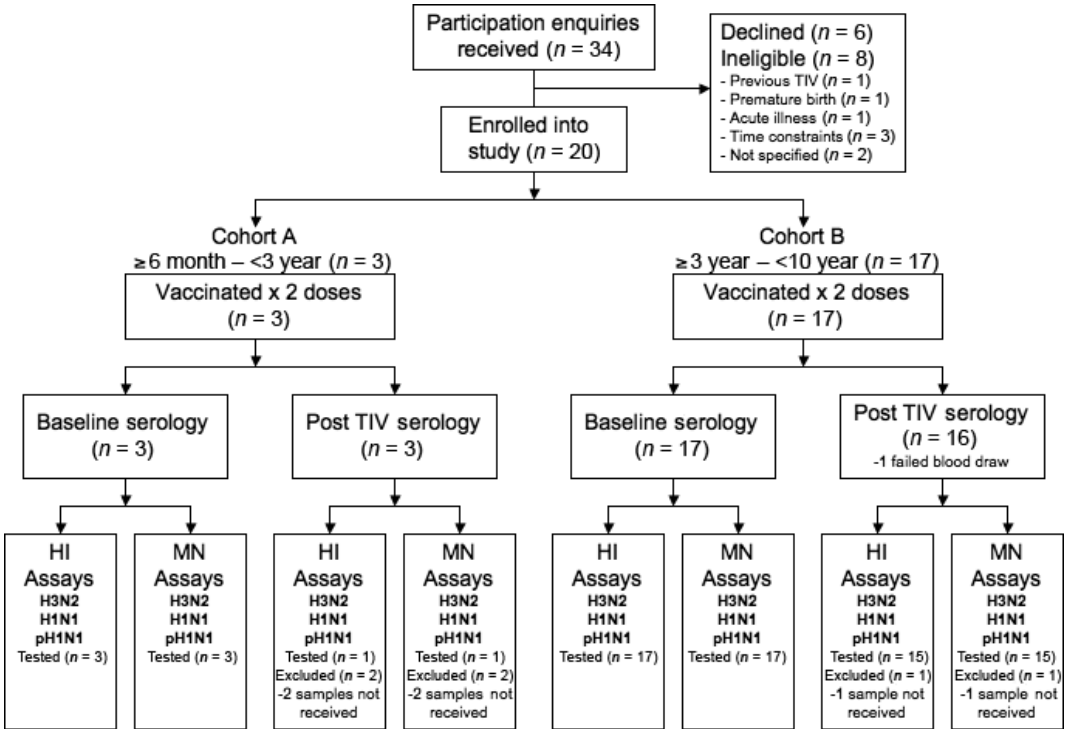

Figure 1. Flowchart of participation in the study, with description of samples available for the analysis in assays against the vaccine strains (H3N2, H1N1) and influenza A (H1N1) 2009 (pH1N1).

Table 1. Antibodies to influenza vaccine strains (H3N2, H1N1) and influenza A (H1N1) 2009 (pH1N1) at baseline and following vaccination with TIV. Median titres and fold increases are reported with interquartile ranges (IQRs)

	Median Titre (IQR)	Seropositive* (%)	Median Fold Increase (IQR)	Seroconversion** (%)
A. HI Titres				
H3N2				
Baseline	30 (20,140)	10/20 (50%)	–	–
Post-TIV	960 (200,1280)	16/16 (100%)	8 (8,16)	16/16 (100%)
H1N1				
Baseline	20 (20,20)	4/20 (20%)	–	–
Post-TIV	40 (40,1040)	13/16 (81%)	2 (2,16)	7/16 (44%)
pH1N1				
Baseline	20 (20,20)	1/20 (5%)	–	–
Post-TIV	20 (20,20)	2/16 (13%)	1 (1,1)	1/16 (6%)
B. MN Titres				
H3N2				
Baseline	25 (10, 160)	10/20 (50%)	–	–
Post-TIV	1280 (320, 1280)	16/16 (100%)	8 (8,24)	15/16 (94%)
H1N1				
Baseline	10 (10,10)	4/20 (20%)	–	–
Post-TIV	160 (80,1120)	15/16 (94%)	12 (5,30)	15/16 (94%)
pH1N1				
Baseline	10 (10,10)	2/20 (10%)	–	–
Post-TIV	10 (10,10)	1/16 (6%)	1 (1,1)	0/16 (0%)

*Seropositivity defined as an HI or MN titre ≥40.
**Seroconversion defined as at least four-fold rise in titre post-vaccination.

of these specimens were collected in August 2009, post-dating the influenza epidemic peak in late June in Melbourne. One child demonstrated a further rise in titre at the second bleed, meeting seroconversion criteria on the MN assay. Another child had a titre of 40 at the post-vaccination visit by HI assay, but <10 on the MN test.

Within-individual correlation between HI and MN assay results was 87% across all viruses, including pre- and post-vaccination sera ($P < 0{\cdot}001$).

Conclusions

This prospective clinical trial demonstrated that seasonal influenza vaccine-naïve children <10 years of age were almost uniformly susceptible to influenza A (H1N1) 2009, assessed according to the serologic correlate of protection of an HI titre of at least 40. Cross-reactive antibody responses to the pandemic strain were not induced by vaccination with the 2009 Southern Hemisphere seasonal TIV formulation, despite evidence of robust immune responses to seasonal H3N2 and H1N1 vaccine antigens in the majority of participants.

A limitation of our study was the small sample size recruited in relation to the initial target, especially participants under 3 years of age. We were, however, successful in recruiting children at the younger end of the Cohort B age spectrum, with a median age of 4 years. The absence of cross-protective antibody responses at baseline in the children tested gives confidence that younger infants would be similarly naïve to the 2009 pandemic H1N1 strain. Our findings were in broad concordance with the low level of cross-reactive antibody reported among similar age groups in a cross-sectional serosurvey conducted in England prior to June 2009[16] and using stored sera in Finland.[17] Somewhat higher levels of baseline seropositivity were reported from the Australian CSL paediatric H1N1 vaccine trial, perhaps reflecting ongoing recruitment for that study beyond the peak of the 2009 influenza A (H1N1) epidemic at several sites, resulting in likely seroconversion following subclinical exposures.[15]

To our knowledge, this study is the first protocol to demonstrate an absence of cross-reactive antibody to influenza A (H1N1) 2009 following administration of the 2009 Southern Hemisphere TIV to children. Studies using stored sera from clinical trials conducted in recent years similarly found that vaccination with seasonal influenza vaccines failed to induce demonstrable cross-protection to the novel strain.[8,9] The likely relevance of these findings to disease risk may be inferred from studies in the naïve ferret model. Pre-administration of matched inactivated pH1N1 vaccines, but not commercially available unadjuvanted seasonal influenza vaccines, resulted in reduced clinical signs and

improved survival following virus challenge.[18] Administration of more immunogenic adjuvanted pH1N1 or seasonal influenza vaccines was associated with improved vaccine-strain-specific protection[19] with some evidence of delayed acquisition and reduced shedding of heterologous strains,[20] without cross-protection against disease.[20]

Our findings of low levels of specific antibody to influenza A (H1N1) 2009 at baseline and following vaccination with TIV are consistent with the observed susceptibility of children to this novel virus. In Melbourne, as elsewhere, children played a key role in fuelling the initial phase of the 2009 winter outbreak.[3–5,7] These data support Australian Government recommendations for the immunisation of children with monovalent A (H1N1) 2009 vaccines both for direct protection and to limit infection transmission beyond the 2009 Southern Hemisphere Influenza season (http://www.healthemergency.gov.au).

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