Short communication

Serological response and persistence in schoolchildren with high baseline seropositive rate after receiving 2009 pandemic influenza A(H1N1) vaccine

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ABSTRACT

The serological response of the current 2009 H1N1 pandemic influenza monovalent vaccine in children exhibiting high baseline seropositive rate was evaluated though a community-based household study. Seroprotection rate of >90% and seroconversion rate of >50% were observed in children one month after receiving the pandemic vaccine. Among children with low baseline antibody titer, a significant lower seroconversion rate (55%) was observed in children who received seasonal trivalent inactivated vaccine (TIV) prior to pandemic vaccine, when compared with those receiving the pandemic vaccine only (86%). Persistence of antibody against the pandemic influenza virus was observed 6 months after vaccination in >80% of children presenting seroprotective antibody levels.

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1. Introduction

In April 2009, a novel influenza A (H1N1) virus that is similar to the influenza viruses previously identified in swine was determined to be the cause of an influenza respiratory illness that spread across North America and was declared a worldwide pandemic by World Health Organization (WHO) in June [1–3]. The 2009 pandemic influenza A (pH1N1) virus contains a novel constellation of gene segments, which most likely stemmed from triple re-assortment of two or more viruses of swine, human, and avian origins [4]. Previous serosurveys have demonstrated little or no cross-protection of the pediatric sera to the pH1N1 virus, which leaves the young children susceptible to infection [5]. For the coming influenza season of 2010–2011 in the post-pandemic period, a safe and effective pH1N1 vaccine for children is urgently needed.

The effectiveness of influenza vaccination in children, in reducing infections and transmission among household members and the community, has been well documented [6–9]. Several modeling analyses indicate that targeted mass immunization of children will contribute to the optimal control and prevention of pandemic and seasonal influenza [10–12]. During the past influenza season of 2009–2010, many governments and vaccine makers began to produce vaccines against the pH1N1 virus in massive quantity. The results from clinical trials reported in US, Europe, China, and Taiwan suggested that a good immunogenicity was generated after one or two doses of vaccine were administered [13–20]. However, the evaluation of antibody response after vaccination in community settings with high baseline seropositivity rate is still lacking.

Moreover, in light of the inadequate cross-protection from either seasonal H1N1 (sH1N1) or pH1N1, the Advisory Committee on Immunization Practice (ACIP) from United States Centers for Disease Control and Prevention (US-CDC) recommended the simul-

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taneous usage of the trivalent influenza vaccine (TIV) for increased protection against the circulating seasonal influenza viruses [21]. Different policies on how TIV was administered in combination with the pH1N1 monovalent vaccine were adopted by different countries. The vaccination policy implemented in Taiwan was to administer pH1N1 monovalent vaccine one month after the TIV vaccination in schoolchildren.

During the last winter influenza season in 2009–2010, we carried out a community-based sero-epidemiological study in central Taiwan to evaluate the antibody response after the pH1N1 vaccination in school children with or without prior receiving TIV immunization. Also, the immune status was followed 6 months after the vaccination to ascertain the decline of the antibody in order to provide the baseline immunity for the immunization program to be implemented during the coming influenza season of 2010–2011.

2. Materials and methods

2.1. Study design and subjects

A sero-epidemiologic study was conducted by the Center for Infectious Disease Education and Research (CIDER) influenza research group in China Medical University (CMU) which was designed to investigate household transmission and vaccine efficacy. The subjects from households with schoolchildren in central Taiwan were recruited and their serum samples were taken by trained nurses during three sampling periods: pre-season (baseline, September 2009–November 2009), post-vaccination (sampling for schoolchildren only, December 2009–February 2010), and post-season (March 2010–July 2010). The demographic characteristics, family contact patterns, and adverse effects after seasonal and pandemic vaccinations were obtained through questionnaires during three household visits. Written informed consent form approved by the CMU Hospital Institutional Review Board (DMR96-IRB-216) was signed by each subject or their parent/guardian.

To evaluate the immune response after pandemic vaccination, 225 subjects with paired serum samples (baseline and post-vaccination) were selected initially. Subjects eligible for data analysis were screened with the following exclusion criteria: (1) age more than 14 years old; (2) incomplete pandemic or seasonal vaccination records; (3) received seasonal vaccine 2 weeks or more before baseline sampling; (4) received the second dose of pandemic vaccine 2 weeks or more before post-vaccination sampling. Finally, data of 193 eligible subjects (from 162 households) of age 5–13 was analyzed (Fig. 1). Among these children, 131 received pandemic vaccine only (denoted by Group 1) and 62 received both pandemic and seasonal vaccines (denoted by Group 2). Each vaccination group was further divided by age/grade into grade 1–3 and grade 4–6. The basic demographic data of the 193 subjects, including age, gender, and grades of the school from both groups, is described in Table 1.

2.2. Vaccine

The 2009 pH1N1 virus vaccine (AdimFluS, A/H1N1) used in this study was produced by Adimmune (Taichung, Taiwan) in embryonated chicken eggs using standard techniques for the production of seasonal inactivated influenza vaccines. It is a monovalent, unadjuvanted, inactivated, thimerosal-preserved, split-virus vaccine. The seed virus was supplied by the US-CDC and prepared from the reassortant vaccine virus NYMC-179A derived from the A/California/7/2009 (H1N1) virus. The vaccine contained 30 µg of hemagglutinin (HA) per milliliter.
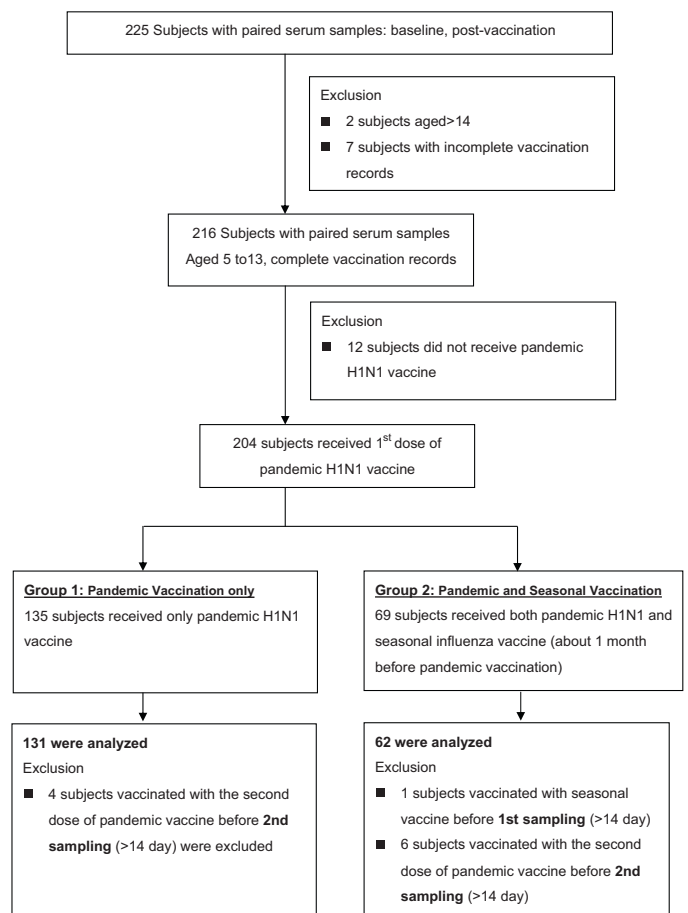

Fig. 1. Enrollment chart of the study subjects.

2.3. Assessment of safety

A questionnaire pertaining to seasonal and/or pandemic vaccination and the corresponding adverse effects within 2 weeks after vaccination was completed by the subject or the guardian. The adverse effects, including redness, swelling or soreness at injection site, dizziness, hoarseness, sore throat, cough, fever ($\geq$38 °C), or any other influenza-like symptoms, were retrospectively evaluated during the month after seasonal and pandemic vaccination were administered.

2.4. Laboratory assays

Antibody titers were measured by a hemagglutination inhibition (HI) assay following the standard protocol by the WHO [22]. The 2009 pH1N1 virus (AdimFluS, A/H1N1) vaccine strain was used to evaluate the immune response after the monovalent pH1N1 vaccine vaccination. The wild-type virus strain used was originally isolated from patient infected by S-OIV H1N1, which is antigenically and genetically closely related to A/California/07/2009. To evaluate the antibody response against TIV, the vaccine strains selected for 2009–2010 northern hemisphere winter season of H1N1 (A/Brisbane/59/2007), H3N2 (A/Brisbane/10/2007) and B (B/Brisbane/60/2008) were also used. All viruses used in this study were cultured from Madin–Darby canine kidney (MDCK) cells and centrifuged at 1600 rpm, 4 °C to remove cell debris. For the HI assay, serum samples were pre-treated with receptor destroying enzyme and titrated in 2-fold dilutions in phosphate-buffered saline (PBS) with an initial dilution of 1:10 and a final dilution of 1:1024. Titers were expressed as the reciprocal of the highest dilution of serum

Table 1
Demographic characteristics of the subjects.

	Group1: pandemic vaccination only (N = 131)	Group2: pandemic and seasonal vaccination (N = 62)	All (N = 193)
Age, years			
Mean ± SD	10.39 ± 1.82	10.03 ± 1.38	10.27 ± 1.7
Median (Q1–Q3)	11 (9–12)	10 (9–11)	10 (9–12)
Range	5–13	8–13	5–13
Gender, no.(%)			
Male	58 (44.27)	27 (43.55)	85 (44.04)
Female	73 (55.73)	35 (56.45)	108 (55.96)
Grader, no.(%)			
1–3	50 (38.17)	28 (45.16)	78 (40.41)
4–6	81 (61.83)	34 (54.84)	115 (59.59)
Pre-vaccination antibody titer, no. (%)			
pH1N1 vaccine strain			
HAI < 1:10			
Grade 1–3	0 (0.0)	0 (0.0)	0 (0.0)
Grade 4–6	7 (8.6)	1 (2.9)	8 (7.0)
HAI ≥ 1:40			
Grade 1–3	43 (86.0)[*]	20 (71.4)	63 (80.8)
Grade 4–6	45 (55.6)[*]	22 (64.7)	67 (58.3)
Seasonal H1N1 vaccine			
HAI < 1:10			
Grade 1–3	1 (2.0)	3 (10.7)	4 (5.1)
Grade 4–6	5 (6.2)	2 (5.9)	7 (6.1)
HAI ≥ 1:40			
Grade 1–3	44 (88.0)	21 (75.0)	65 (83.3)
Grade 4–6	68 (84.0)	29 (85.3)	97 (84.4)
Seasonal H3N2 vaccine			
HAI < 1:10			
Grade 1–3	0 (0.0)	0 (0.0)	0 (0.0)
Grade 4–6	0 (0.0)	0 (0.0)	0 (0.0)
HAI ≥ 1:40			
Grade 1–3	50 (100.0)	28 (100.0)	78 (100.0)
Grade 4–6	78 (96.3)	33 (97.1)	111 (96.5)

[*] $p < 0.05$.

where hemagglutination was prevented. Samples that were negative by HI were assigned a titer of 1:5 for computational purposes in obtaining a 4-fold increase of HI titers. Seroconversion was defined as either a pre-vaccination titer of <1:10 together with a post-vaccination titer of ≥1:40, or a significant increase in HI titer by a factor of 4 or greater. Seroprotection was defined as HI titer of 1:40 or more.

2.5. Statistical analysis

The immunogenicity outcomes, including seroconversion rate, seroprotection rate, and geometric mean titer (GMT) ratio, were evaluated based on HI titers. HI titer below the detection limit (1:10) was assigned a titer of 1:5 in order to compute the GMT. The GMT ratio was calculated by dividing the post-vaccination GMT by the baseline GMT.

The point estimates and 95% confidence intervals (CI) of the immunogenicity outcomes were calculated using generalized estimating equations (GEE) to account for household correlation. The comparisons were made between the vaccination group (Group 1 vs. Group 2), age/grade group (grade 1–3 vs. grade 4–6), and baseline HI titer (<40 vs. ≧40). A p-value of less than 0.05 represented statistically significance. All statistical analyses were performed using SAS statistical software version 9.2 (SAS Institute, Cary, NC).

3. Results

3.1. Immunogenicity to the monovalent pH1N1 vaccine

Prior to the vaccination, more children (86%) in grade 1–3 had high HI antibody titers of 1:40 or more against pandemic vaccine strain than those in grade 4–6 (55.6%) among Group 1 (Table 1),

but less so among children in Group 2 for grade 1–3 (71.4%) as compared to grade 4–6 (64.7%). No significant difference in HI antibody titer against seasonal H1N1 or H3N2 vaccine strains was found between age/grade groups prior to vaccination. After vaccination, seroprotective response (HAI titer ≥ 40) was observed in 96.0% and 96.4% in schoolchildren of grade 1–3 from Group 1 and 2, respectively. Similarly, the seroprotective response was observed in 92.6% and 85.3% of children of grade 4–6 among Groups 1 and 2 (Table 2), respectively.

In addition to the proportion of study subjects having HI titers higher than 1:40, we also compute the seroconversion rates. Age-related differences were found among subjects receiving the monovalent pH1N1 vaccine, with statistically significantly higher ($p < 0.05$) seroconversion rates (70.4%; 95% CI, 59.3–79.4%) in subjects who were in grade 4–6 than those in grade 1–3 (38%; 95% CI, 25.8–51.9%) in Group 1. Meanwhile, having received TIV prior to pH1N1 vaccination increased the seroconversion rate in grade 1–3 up to 57.1% (95% CI, 38.7–73.8%) when compared with the seroconversion rate of 38% for children with monovalent pH1N1 vaccination only in Group 1 (Table 2).

Consistent with the age-related seroconversion rate after vaccination, the increase in GMT ratio among subjects of grade 4–6 in Group 1 was 6.3-fold, which was significantly higher than 2.3-fold among those in grade 1–3 in Group 2. Extra dose of TIV resulted in 3.4-fold and 3.9-fold increase in GMT ratio among children of grade 1–3 and 4–6 in Group 2, respectively. However, no statistical significance in fold increase of GMT ratio was observed between Group 1 and Group 2. Fig. 2 illustrates that the reverse cumulative percentage of subjects having different levels of HI titers against the 2009 pH1N1 virus before and after vaccinations in the different study groups. Again, a single vaccination results in strong antibody responses to pH1N1 vaccine strain in the grade 4–6 group.

Table 2
Immune responses after the 2009 pandemic Influenza A H1N1 vaccination in group1 (pandemic vaccine only) and Group 2 (pandemic and seasonal vaccine).

	Group 1: pandemic vaccination only				Group 2: pandemic and seasonal vaccination			
	GMT (95% CI) baseline	GMT ratio (95% CI)	Seroprotection rate (95% CI)	Seroconversion rate (95% CI)	GMT (95% CI) baseline	GMT ratio (95% CI)	Seroprotection rate (95% CI)	Seroconversion rate (95% CI)
Pandemic H1N1-vaccine strain								
Grade 1–3	47.9 (40.4–56.9)[*]	2.3 (1.6–3.3)[**]	96.0 (85.3–99.0)	38.0 (25.8–51.9)[**]	45.3 (34.4–59.6)	3.4 (2.2–5.4)	96.4 (78.6–99.5)	57.1 (38.7–73.8)
Grade 4–6	30.4 (24.7–37.5)[*]	6.3 (4.5–9.0)[**]	92.6 (84.5–96.6)	70.4 (59.3–79.4)[**]	36.1 (27.8–46.9)	3.9 (2.3–6.7)	85.3 (69.0–93.8)	52.9 (35.7–69.5)
Total	36.2 (31.1–42.1)	4.3 (3.3–5.7)	93.9 (88.2–96.9)	58.0 (49.2–66.4)	40.0 (32.7–48.9)	3.7 (2.5–5.4)	90.3 (80.1–95.6)	54.8 (40.8–68.1)
Seasonal H1N1-vaccine strain								
Grade 1–3	102.7 (76.7–137.4)	3.6 (2.6–5.0)	100 (92.9–100)[a]	50.0 (37.0–63.0)	60.9 (36.6–101.5)	4.9 (2.7–8.9)	100 (87.9–100)[a]	53.6 (35.4–70.8)
Grade 4–6	95.7 (71.8–127.7)	3.3 (2.5–4.4)	97.5 (90.6–99.4)	50.6 (40.0–61.2)	98.1 (65.3–147.3)	2.7 (1.8–4.0)	100 (89.9–100)[a]	38.2 (24.4–54.2)
Toal	98.3 (79.3–121.9)	3.4 (2.7–4.2)	98.5 (94.1–99.6)	50.4 (41.9–58.9)	79.1 (56.4–110.9)	3.5 (2.5–5.1)	100 (94.2–100)[a]	45.2 (33.5–57.3)
Seasonal H3N2-vaccine strain								
Grade 1–3	249.3 (197.8–314.3)	2.1 (1.6–2.8)	100 (92.9–100)[a]	40.0 (27.0–54.6)	256.1 (158.4–414.1)	2.2 (1.4–3.3)	100 (87.9–100)[a]	39.3 (23.3–58.0)
Grade 4–6	161.4 (125.9–206.9)	1.6 (1.3–2.1)	100 (95.5–100)[a]	28.4 (20.1–38.5)	188.3 (122.1–290.6)	1.5 (1.0–2.2)	97.1 (81.8–99.6)	26.5 (14.6–43.2)
Toal	190.5 (158.4–229.1)	1.8 (1.5–2.2)	100 (97.2–100)[a]	32.8 (25.4–41.2)	216.4 (156.5–299.2)	1.8 (1.4–2.3)	98.4 (89.4–99.8)	32.3 (21.8–44.8)

[a] The 95% confidence intervals were calculated using Wilson score method for proportion when the value of rate is 100%.
[*] denoting $p < 0.01$ of significant difference between Grade 1–3 and Grade 4–6 among Group 1 using the GEE approach.
[**] denoting $p < 0.001$ of significant difference between Grade 1–3 and Grade 4–6 among Group 1 using the GEE approach.

3.2. Immunogenicity to the TIV vaccine

In Group 2, TIV vaccination prior to receiving the pandemic vaccine induced strong immune response not only against the A/california/2009 H1N1-like NYMC-179A antigen, but also against seasonal influenza A/H1N1, A/H3N2, and B antigens in both age groups. The seroconversion rate of seasonal influenza A/H1N1 and A/H3N2 was slightly lower in children of grade 4–6 (38.2% and 26.5%, respectively) than in grade 1–3 (53.6% and 39.3%, respectively). Similarly, the GMT ratio was also lower in grade 4–6 than in grade 1–3. However, subjects from both age/grade groups in Group 2 attained nearly 100% seroprotection rate against both seasonal influenza A/H1N1 and B/H3N2 vaccine strains post-vaccination (Table 2).

Interestingly, receiving pandemic vaccine alone also induced comparable immune response against seasonal influenza A/H1N1 and A/H3N2 antigens in Group 1. The seroconversion rate of seasonal influenza A/H3N2 was slightly lower in the children of grade 4–6 (28.4%) than in grade 1–3 (40.0%) but it was about equivalent (50%) to seasonal influenza A/H1N1 for both grades. Although a slightly lower GMT ratio were observed in grade 4–6 (1.6-fold) than in grade 1–3 (2.1-fold), nearly 100% seroprotection rate was also achieved in both age groups post vaccination as shown in Group 1 (Table 2).

3.3. Baseline titer of pH1N1 vaccine strain and immunogenicity

Among children of grade 4–6 with baseline HI titer <40, the seroconversion rate was higher in Group 1 (88.9%; 95% CI, 74.3–95.7%) than that in Group 2 (50.0%; 95% CI, 24.4–75.6%) with statistical significance ($p < 0.05$) (Table 3). The seroconversion rate was also higher in children with baseline HI titer <40 in Group 1 (86.0%; 95% CI, 72.5–93.5%) than in Group 2 (55.0%; 95% CI, 33.6–74.7%) with statistically significance ($p < 0.05$). Furthermore, the subjects in Group 1 with higher baseline titer (HI ≥ 1:40) showed lower seroconversion rate of 32.6% and 55.6% for children in grades 1–3 and 4–6, respectively, when compared with the subjects from the same

group with lower baseline titer (HI < 1:40) where the seroconversion rates were 71.4% and 88.9% for children in grades 1–3 and 4–6, respectively.

3.4. Side effects after vaccination

There were in total 17 events of adverse effects reported by the children in our study after receiving the vaccines. Among the symptoms reported, fever (3.6%) and any other influenza-like symptom (4.7%) were the most common adverse events. In particular, fever was observed more frequently in children of grade 1–3 (10%) than in children of grade 4–6 (1.2%) after the monovalent pH1N1 vaccination in Group 1 ($p = 0.049$) (Table 4).

3.5. Persistence of the immunity

The children were followed for more than 6 months after the vaccination and a significant high proportion of the children retained a HI titer of 1:40 or higher, which confers protection from infection by the novel 2009 influenza virus (pH1N1). 94.4% of the children in grade 1–3 and 92.6% of the grade 4–6 children in Group 1 have HI antibody levels that confer seroprotection. Similarly, 100% of the children in grade 1–3 and 82.4% of the grade 4–6 children in Group 2 had HI antibody level conferring seroprotection.

4. Discussion

This community-based study was conducted with children who might have been previously infected by pH1N1 influenza virus and approximately 65% of them had HI antibody of 1:40 or higher before vaccination, which distinguishes our study from other clinical trial studies with subjects having low pre-vaccination antibody titer and hence the resulting immune response might be different. To our best knowledge, the pandemic vaccine immunogenicity has never been evaluated on a community-based population. Our results are consistent with the results of previous studies that Adimmune 2009 monovalent pH1N1 influenza vaccine is immunogenic and
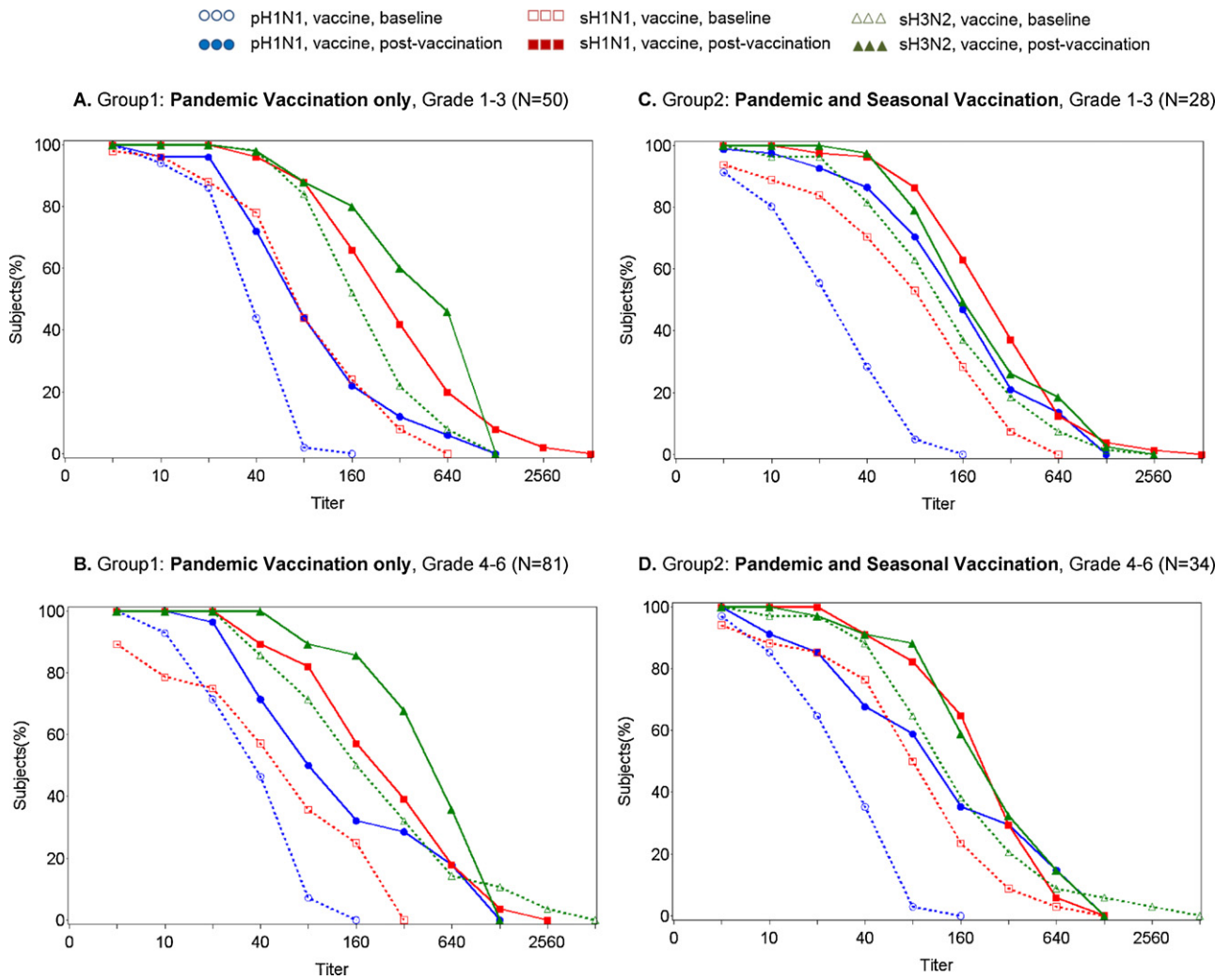
○○○ pH1N1, vaccine, baseline
●●● pH1N1, vaccine, post-vaccination
□□□ sH1N1, vaccine, baseline
■■■ sH1N1, vaccine, post-vaccination
△△△ sH3N2, vaccine, baseline
▲▲▲ sH3N2, vaccine, post-vaccination

A. Group1: **Pandemic Vaccination only**, Grade 1-3 (N=50)

B. Group1: **Pandemic Vaccination only**, Grade 4-6 (N=81)

C. Group2: **Pandemic and Seasonal Vaccination**, Grade 1-3 (N=28)

D. Group2: **Pandemic and Seasonal Vaccination**, Grade 4-6 (N=34)

Fig. 2. Reverse cumulative distribution curves of the hemagglutination inhibition (HI) antibody titers against different strains, according to vaccination group and age/grade group.

safe in children [19,23], and the present pandemic influenza vaccine after only one dose induces immune responses that meet all international licensing criteria applicable for the children with high baseline antibody titer [14,24].

The immune response observed after a single pandemic monovalent pH1N1 vaccination in this study showed high seroprotection rates of 96% and 92.6% of among children of grade 1–3 and 4–6,

respectively, which is different from the immune response exhibited in previous studies of seasonal H1 or novel H5N1 strain in vaccine-naïve children [17,25], as well as in previous vaccination studies of children with low pre-vaccination antibody titers in which two doses are required to induce protective responses in children aged under 9 [19,20]. A possible reason for this apparently high immunogenic response of the current vaccine could be that

Table 3
Seroconversion rate among those with baseline hemagglutination inhibition (HI) titer <40 and baseline HI titer ≥40.

Seroconversion rate (95% CI)	Baseline HI < 40		Baseline HI ≥ 40	
	Group1: pandemic vaccination only	Group2: pandemic and seasonal vaccination	Group1: pandemic vaccination only	Group2: pandemic and seasonal vaccination
Pandemic H1N1-vaccine strain				
Grade 1–3	71.4 (32.7–92.8)	62.5 (28.5–87.5)	32.6 (20.5–47.5)[*]	55.0 (33.6–74.7)
Grade 4–6	88.9 (74.3–95.7)[†,‡]	50.0 (24.4–75.6)[†]	55.6 (40.4–69.7)[*,‡]	54.5 (33.4–74.1)
Total	86.0 (72.5–93.5)[¶,§]	55.0 (33.6–74.7)[¶]	55.0 (33.6–74.7)[§]	54.8 (38.1–70.4)

[*] denoting $p < 0.05$ for significant difference between Grade 1–3 and Grade 4–6 among Group1 with baseline HI titer ≥40 using the GEE approach.
[¶] denoting $p < 0.01$ for significant difference between Group1 and Group 2 among all subjects with baseline HI titer <40 using the GEE approach.
[†] denoting $p < 0.01$ for significant difference between Group1 and Group 2 among Grade 4–6 with baseline HI titer <40 using the GEE approach.
[§] denoting $p < 0.0001$ for significant difference between all subjects with baseline HI titer <40 and baseline HI titer ≥40 within Group1 using the GEE approach.
[‡] denoting $p < 0.0001$ for significant difference between Grade 4–6 with baseline HI titer <40 and baseline HI titer ≥40 within Group1 using the GEE approach.

Table 4
Adverse effects after the 2009 pandemic influenza A H1N1 vaccination, according to vaccination group and age/grade group.

Adverse effects, no. (%)	Group 1: pandemic vaccination only		Group 2: pandemic and seasonal vaccination		All (N = 193)
	Grade 1–3 (N = 50)	Grade 4–6 (N = 81)	Grade 1–3 (N = 28)	Grade 4–6 (N = 34)	
Any symptom	7 (14.0)	6 (7.4)	1 (3.6)	3 (8.8)	17 (9.7)
Redness, swelling or soreness at injection site	0 (0.0)	1 (1.2)	0 (0.0)	2 (5.9)	3 (1.6)
Dizziness	0 (0.0)	3 (3.7)	0 (0.0)	0 (0.0)	3 (1.6)
Hoarseness	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Sore throat	3 (6.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.6)
Cough	3 (6.0)	1 (1.2)	0 (0.0)	0 (0.0)	4 (2.1)
Fever (≥38 °C)	5 (10.0)*	1 (1.2)*	1 (3.6)	0 (0.0)	7 (3.6)
Other symptom	5 (10.0)	3 (3.7)	0 (0.0)	1 (2.9)	9 (4.7)

* Notation denoting significant difference between Grade 1–3 and Grade 4–6 among Group1 using the GEE approach: $p < 0.05$.

a high proportion of children in the community had been previously exposed to this novel pH1N1 virus, which primed the immune response before the vaccination.

Our study found that there is a diminution of antibody response in those with higher baseline antibody titer and having receiving TIV vaccination one month before. This result differs from the results of a previous study where TIV and pH1N1 monovalent vaccine were administered simultaneously [13]. No immune interference was observed as the immune response, measured by the GMT ratio, seroconversion rate, and seropositivity rate, was at similar levels in groups either receiving pandemic vaccine only or co-administered pandemic and seasonal vaccine. The mean durations between receiving a pH1N1 vaccination to the collection of post-vaccination sera of the subjects in the two groups were calculated, no difference was found among the subjects in Group 2 (mean ± standard deviation (sd): 24.9 ± 15.3) and in Group 1 (mean ± sd: 24.2 ± 12.4). Therefore, the significant lower pH1N1 seroconversion is less likely to come from inadequate time allowed for full antibody response. Also, the recruitment of children into this study started during September after the school began and the status of the children receiving seasonal TIV or pandemic H1N1 vaccine was unknown. Although the study is not randomized, there was no other factor for determining whether the children were being assigned to Group 1 or 2. Similar result was also observed in other studies [17], and it is intuitively plausible that immune interference was in effect while two similar vaccines were administered within one month.

Our findings pertaining to antibody response against seasonal H1N1 and H3N2 vaccine strains in the group of children receiving only pandemic H1N1 monovalent vaccine was surprising. Respective seroconversion rates of 50.4% and 32.8% to seasonal H1N1 and H3N2 vaccine strains suggest that the wild-type influenza virus, especially H3N2, might have co-circulated in the community, as co-circulation of the 2009 pandemic and seasonal strains had also been reported elsewhere [26]. Without virological confirmation, our results on the seroconversion rate of H3N2 vaccine strain observed in children not receiving TIV and had few clinical symptoms raise the question that children might acquire an asymptomatic or subclinical infection and perhaps play a significant role as the major disseminators in the spread of influenza [27,28]. These findings support the belief that intervention strategy targeting schoolchildren could be more efficacious [10].

Finally, persistence of immunity was also evaluated. During the follow-up of the children in the study 6 months after vaccination, a significantly high proportion of the children retained a HI titer of 1:40 or higher against pH1N1 virus. The serum protection rate of higher than 90% in children should provide sufficient herd immunity for this coming influenza season of 2010–2011. Mass influenza immunization program, such as the one implemented in Taiwan since 2007 targeting schoolchildren of age 9 or under, offers repeated immunization which could enhance the antibody titer, preventing infection in the children as well as reducing morbid-

ity and mortality in the elderly, as previous studies have suggested [7,8].

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