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Optimal vaccination strategies for 2009 pandemic H1N1 and seasonal influenza vaccines in humans☆

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ABSTRACT

A randomized clinical trial was conducted to assess whether the immunogenicity of seasonal and pandemic (H1N1/09) influenza vaccines is affected by the order of vaccine administration. 151 healthy adult volunteers were randomized into three groups. All groups received one dose (15 μg haemagglutinin) each of a pandemic H1N1 vaccine and a seasonal trivalent vaccine. Group 1 received the pandemic H1N1 vaccine first, followed by the seasonal vaccine 21 days later. Group 2 received vaccinations in vice versa and Group 3 received both vaccines simultaneously. Post-vaccination blood samples were collected to determine the immunogenicity by hemagglutination-inhibition (HI), microneutralization (MN), and B cell ELISPOT assays. All three vaccination strategies were well-tolerated and generated specific immune responses. However, we found a significant difference in magnitude of antibody responses to pandemic H1N1 between the three groups. Pre- or co-vaccination with the seasonal flu vaccine led to a significant reduction by 50% in HI titre to pandemic H1N1 virus after pandemic vaccination. Pre- or co-vaccination of pandemic H1N1 vaccine had no effect on seasonal flu vaccination. MN and ELISPOT assays showed a similar effect. Vaccination with pandemic H1N1 vaccine first is recommended to avoid an associated inhibitory effect by the seasonal trivalent flu vaccine. Clinical_Trials identifier: NCT01008137.

1. Introduction

The novel swine-origin influenza A (H1N1) 2009 virus that emerged in North America has caused pandemic influenza as declared by the World Health Organization (WHO) on June 11, 2009. Mass vaccination will be crucial to control this pandemic. Pandemic H1N1 vaccine trials have been carried out globally and there is general agreement that one dose containing 15 μg haemagglutinin without adjuvant is sufficient to trigger immune responses required for licensing in a population aged 3–60 years [1–4].

While global vaccination against the pandemic H1N1/09 virus is being implemented, the need for annual seasonal flu vaccines remains. However, it is not known whether the two vaccines affect each other in terms of safety and immunogenicity, or whether the order of administration is important.

In this study, we conducted a randomized, observer-masked clinical trial in healthy adults to compare three possible immunization schedules using a licensed pandemic split vaccine and a seasonal trivalent split influenza vaccine recommended for the 2009–2010 season. The pandemic vaccine was administered before, after or at the same time as the seasonal vaccine. The safety and immunogenicity of both vaccines were evaluated by three independent assays.

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2. Materials and methods

2.1. Study design

Between 24th October and 6th December, 2009, a prospective, randomized, observer-blind, parallel-group clinical trial was conducted at a single site in Chaoyang District, Beijing, China. The purpose of the study was to evaluate the effect of seasonal influenza vaccine on immunogenicity and safety of the 2009 pandemic H1N1 vaccine in healthy adults between the ages of 18 and 60 years in a two-dose regimen. All subjects provided written informed consent.

A randomization list was prepared by a statistician, employed by Sinovac Biotech Co., Ltd. who was not involved in the rest of the trial. The randomization code was provided in a sealed envelope to the vaccine administrator. All subjects and investigators were masked from the assignments. The study complied with Good Clinical Practice guidelines and the Declaration of Helsinki. The protocol was approved by Beijing Centre for Disease Control and Prevention and the ethics review committee. The study was registered with ClinicalTrials.gov, number NCT01008137.

2.2. Vaccine

The vaccines used in this study were manufactured by Sinovac Biotech Co., Ltd. (Beijing, China). The 2009 pandemic H1N1 vaccine was a monovalent, unadjuvanted, split vaccine. The seed virus was prepared from the reassortant vaccine virus NYMC X-179A (New York Medical College, New York), derived from the A/California/7/2009 (H1N1) virus, the candidate reassortant vaccine virus for pandemic H1N1 recommended by the WHO. The vaccine was prepared in embryonated chicken eggs using standard techniques used for the production of seasonal trivalent inactivated vaccine as described previously [4]. Each dose contained haemagglutinin in 15 μg/0.5 ml/vial.

The virus strains in the seasonal vaccine were high growth reassortants of A/Brisbane/59/2007(H1N1), A/Brisbane/10/2007(H3N2), and B/Brisbane/60/2008 virus, according to the WHO recommended composition of influenza virus vaccines for use in the 2009–2010 northern hemisphere influenza season (http://www.who.int/csr/disease/influenza/recommendations2009_10north/en/index.html). As with most licensed trivalent inactivated seasonal influenza vaccines, it contained 15 μg haemagglutinin per strain per dose.

2.3. Study and safety procedures

Healthy, non-pregnant adults between the ages of 18 and 60 years were eligible for enrolment. Exclusion criteria included subjects with confirmed or suspected 2009 H1N1 infection and history of pandemic H1N1 or seasonal influenza vaccines during the preceding 6 months.

A total of 151 eligible subjects were randomized and assigned into 3 groups in a 1:1:1 ratio. Subjects were immunized in a predesignated order provided by a statistician who was not involved in the rest of the trial. Vaccinations were done by personnel who did not take part in the subsequent assessment of safety and immunogenicity, thus maintaining the masking. Group 1 received 2009 pandemic H1N1 vaccine on day 0 and seasonal vaccines on day 21. Group 2 received seasonal vaccines on day 0 and pandemic H1N1 vaccine on day 21. Group 3 received both pandemic H1N1 and seasonal vaccines on day 0. Each dose was administered intramuscularly into the deltoid muscles of the left or right arm separately.

2.4. Safety assessments

After an on-site safety observation of 30-min duration, subjects were requested to record underarm body temperature, any injection-site and systemic reactions on diary cards. For three days after each immunization, any local adverse events at the injection site and systemic adverse events were recorded. Investigators determined the diameter of any erythemas, swelling, indurations and rashes. Daily temperatures were recorded by self-reporting in diary cards. All adverse events were graded using standard scale (http://www3.niaid.nih.gov/LabsAndResources/resources/DMIDClinRsrch/toxtables.html).

2.5. Laboratory assays

For each subject, 20–30 ml heparin-containing blood and 3–5 ml serum samples were collected by venipunture on day 0, 7 and 21 days after each immunization thus all subjects were bled on day 0, 7, 21, 28 and 42 days after the first immunization, except for Group 3 which received no 2nd immunization on day 21 and hence no 7 day follow-up for this vaccination at day 28.

The immunogenicity of the pandemic H1N1 and seasonal influenza vaccines in the serum samples was evaluated by haemagglutination-inhibition (HI), microneutralization (MN) and B-cell ELISPOT assays [5]. The assays were performed with antigens or viruses from A/California/7/2009(H1N1pdm), A/Brisbane/59/2007(H1N1) and A/Brisbane/10/2007(H3N2).

2.6. Statistical analysis

A sample size of minimum 50 subjects per study group was chosen pragmatically following the European Union Committee for Medicinal Products for Human Use (CHMP—http://www.ema.europa.eu) guidelines on harmonization and requirements of influenza vaccine (CHMP/BWP/214/96) and immune interference on vaccine (EMEA/CHMP/VWP/164653/2005). Also, this scale would facilitate the early completion of this study of public health priority. All data analyses were done according to a pre-established analysis plan by an independent statistician. The incidence of adverse events was based on the most severe response, expressed in terms of the number and proportion of individuals who had adverse events. The safety data were summarized descriptively.

Immunological endpoints were based on HI licensure criteria established by the CHMP and included geometric mean titre (GMT), post-to-pre-vaccination GMT ratio, seroconversion rate and seropositivity rate (ref CHMP/BWP/214/96). Seroconversion rate was defined as the percentage of subjects that had a titre before vaccination of less than 1:10 and a titre after vaccination of 1:40 or more, or a titre before vaccination of 1:10 or more and at least a fourfold increase after vaccination. HI titres $\geq$ 1:40 are considered protective. The HI antibody titres were transformed into logarithmic scale for the calculation of GMT. The distribution of the GMT titre of each group was described with a reverse cumulative distribution curve. The results were summarized with point estimates and two-sided 95%CI. A p value of less than 0.05 was considered significant and all reported p values are two-sided.

Due to the lack of established immune correlates for microneutralization, the proportion of subjects who had seroconverted (an increase in the antibody titre by a factor of 4 or more) and a microneutralization titre of 1:40 or more were assessed for analysis for immunogenecity between different groups. Likewise, the number of antigen-specific antibody secreting cells was expressed as ASC per million PBMC. Differences in HI titre, MN titre and frequency of ASC between groups were tested using non-parametric Kruskal–Wallis's test with post hoc Dunn's test for multiple comparisons.

All graphs are presented using GraphPad Prism software (version 5) and statistical analysis was performed by using SPSS software (version 13.0).

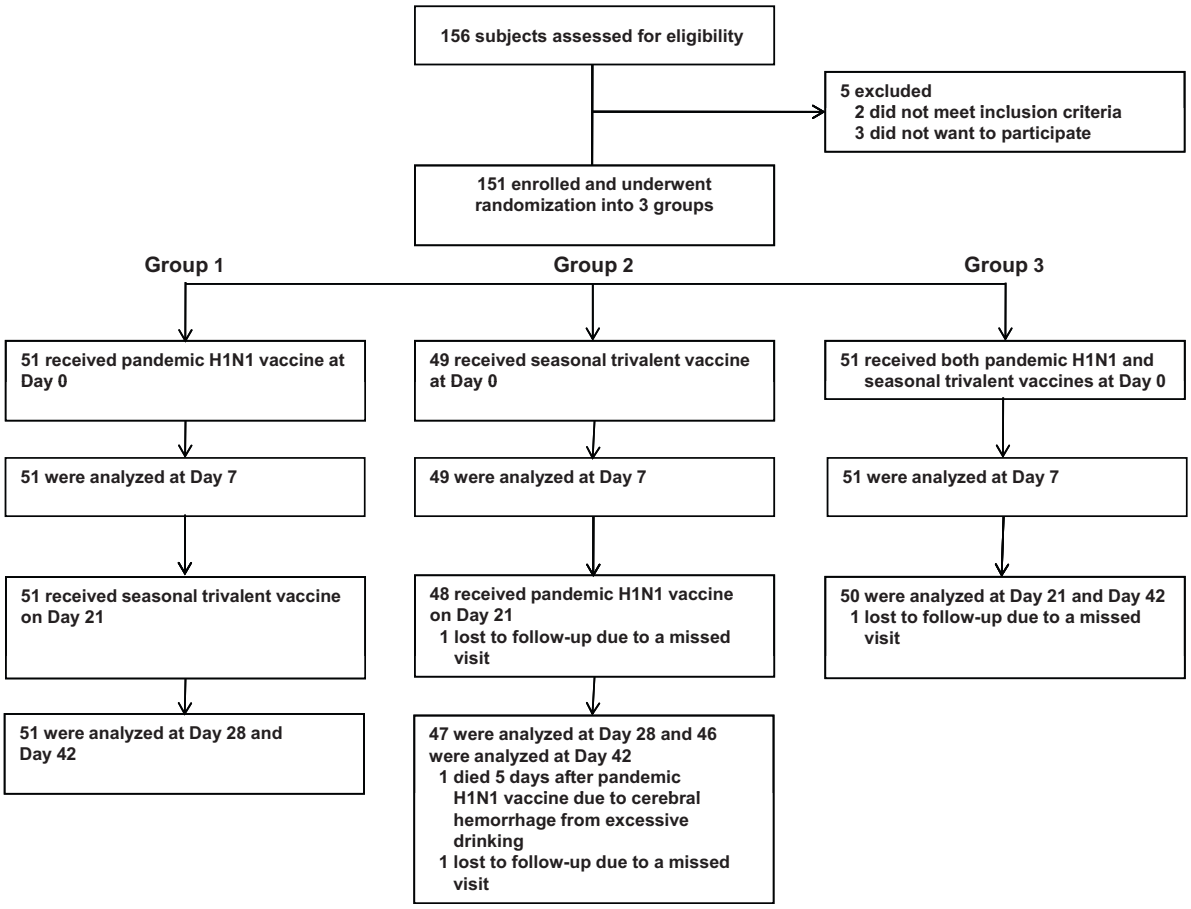

Fig. 1. Enrolment and outcomes.

3. Results

3.1. Study subjects

From 24th October to 6th December, 2009, a total of 156 eligible healthy adult volunteers aged from 18 to 60 years were recruited in the study (Fig. 1). Five were excluded between enrolment and randomization. Thus a total of 151 subjects were randomized into three immunization groups. Subjects in Group 1 received pandemic H1N1 vaccine on day 0 followed by seasonal trivalent vaccine 21 days later. Group 2 subjects received the seasonal trivalent vaccine initially and the pandemic H1N1 at day 21. Group 3 subjects received both vaccines on day 0. All provided blood samples for immunogenicity assays before receiving the first dose on day 0. One subject each of Groups 2 and 3 missed their day 21 visit due to travelling, thus failed to provide blood samples and in the case of the Group 2 individual, failed to receive the second dose. However, both informed their investigators of no medical complaints after the first dose and were included in the first dose tolerability analysis and day 7 immunogenicity test analysis. One subject from Group 2 died five days after the second dose. The cause of death was confirmed as cerebral haemorrhage due to excessive drinking and was unrelated to vaccination as judged by the investigators and relevant medical experts. This subject was included in the first dose tolerability and immunogenicity analysis. Another subject in Group 2 missed the day 42 follow-up due to travelling and thus was excluded from the day 42 analyses.

The demographics of the study population are summarized in Table 1 in the Supplementary Appendix. The mean age ranged from 34.7 to 41.4 years in all three groups but there was a statistically significant difference in age between the three groups ($p = 0.028$, Fisher's exact test) as a chance result of the random stratification. No differences were found in height, weight and sex between the groups.

3.2. Adverse events

No serious adverse events or adverse events of special interest were reported over the 3 study groups (Table 2 in the Supplementary Appendix). No withdrawals resulted from adverse events. After both vaccinations, the proportions of subjects reporting adverse events were 17.6%, 24.5% and 29.4% for Groups 1, 2 and 3 respectively; no significant difference between three groups was found ($p = 0.375$). Most of the adverse reactions were mild in intensity (Group 1, 13.7%; Group 2, 20.4%; Group 3, 25.5%). Pain at the injection site was the most common reaction after the first dose. The main systemic reactions were headache, angina and fatigue; no difference was found between the three groups.

3.3. Immunogenicity

At baseline, 15 of the 151 subjects (10%) had antibody titres of 1:40 or more against pandemic H1N1 virus in haemagglutination-inhibition (HI) assays (Table 1). The level of pre-existing antibody against pandemic H1N1 was comparable to other published studies [2,4]. The proportion of subjects with a baseline antibody titre greater than 1:40 was higher for seasonal H1N1 (63/151, 42%), H3N2 (28/151, 19%) and influenza B (70%).

In all 3 groups, co-immunization or sequential immunization of a single 15 μg dose of the pandemic H1N1 vaccine induced strong humoral immune responses that meet all three European Union Licensing Criteria on 21 days after immunization. Seroconversion

Table 1
Hemagglutination-inhibition antibody response before and after immunization in three groups[a].

Day and antigen	Group 1				Group 2				Group 3			
	GMT (95%CI)	GMT ratio (95%CI)	Seroconversion (95%CI)	Seropositivity (95%CI)	GMT (95%CI)	GMT ratio (95%CI)	Seroconversion (95%CI)	Seropositivity (95%CI)	GMT (95%CI)	GMT ratio (95%CI)	Seroconversion (95%CI)	Seropositivity (95%CI)
Day 0			*N* = 51				*N* = 49				*N* = 51	
Pandemic H1N1	9.1 (7.2–11.5)	–	–	13.7 (6.2–27.0)	8.1 (6.5–10.1)	–	–	10.2 (3.8–23.1)	7.1 (6.0–8.4)	–	–	5.9 (1.5–17.3)
Seasonal H1N1	30.9 (23.6–40.5)	–	–	45.1 (31.4–59.8)	25.4 (19.6–33.0)	–	–	32.7 (20.4–47.8)	28.1 (21.4–36.8)	–	–	49.0 (35.0–63.5)
Seasonal H3N2	12.4 (9.1–17.0)	–	–	25.5 (14.8–40.1)	9.9 (7.5–13.0)	–	–	14.3 (6.4–28.0)	9.9 (7.5–13.0)	––	–	13.7 (6.2–27.0)
Seasonal B	33.5 (28.8–39.0)	–	–	70.6 (56.0–82.5)	36.7 (31.0–43.6)	–	–	71.4 (56.5–83.4)	31.7 (26.9–37.4)	–	–	66.7 (52.0–79.2)
Day 7			*N* = 51				*N* = 49				*N* = 51	
Pandemic H1N1	145.5 (93.6–226.1)	16.0 (10.4–24.6)	76.5 (62.2–87.2)	82.4 (68.6–91.6)	35.7 (26.4–48.3)	4.4 (3.4–5.7)	42.9 (29.1–57.9)	51.0 (36.5–65.6)	112.4 (78.1–161.8)	15.8 (11.1–22.4)	90.2 (77.8–97.0)	90.2 (77.8–97.0)
Seasonal H1N1	54.7 (41.7–71.7)	1.8 (1.4–2.3)	17.6 (8.9–31.5)	74.5 (60.1–85.6)	151.2 (107.1–213.4)	5.9 (4.0–8.9)	57.1 (42.3–71.2)	93.9 (82.1–99.3)	212.8 (143.2–316.3)	7.6 (5.0–11.5)	60.8 (46.1–74.1)	90.2 (77.8–97.0)
Seasonal H3N2	16.3 (11.9–22.4)	1.3 (1.1–1.5)	3.9 (0.7–14.6)	25.5 (14.8–40.1)	87.1 (51.9–146.2)	8.8 (5.3–14.7)	63.3 (48.3–76.5)	73.5 (58.7–85.0)	115.5 (70.7–188.6)	11.7 (7.2–19.0)	64.7 (50.0–77.5)	76.5 (62.2–87.2)
Seasonal B	41.7 (35.9–48.3)	1.2 (1.1–1.4)	7.8 (2.5–19.8)	80.4 (66.5–90.2)	87.1 (67.4–112.5)	2.4 (1.8–3.1)	40.8 (27.3–56.0)	93.9 (82.1–99.3)	92.9 (74.4–116.0)	2.9 (2.3–3.8)	49.0 (35.0–63.5)	96.1 (85.4–100.0)
Day 21			*N* = 51				*N* = 48				*N* = 50	
Pandemic H1N1	640.0 (430.9–950.7)	70.4 (48.8–101.5)	96.1 (85.4–100.0)	98.0 (88.2–100.0)	43.0 (31.0–59.7)	5.4 (4.0–7.4)	52.1 (37.4–66.8)	62.5 (47.3–76.0)	282.5 (198.9–401.2)	39.4 (27.8–55.8)	98.0 (88.0–100.0)	98.0 (88.0–100.0)
Seasonal H1N1	121.9 (90.3–164.5)	3.9 (2.8–5.5)	49.0 (35.0–63.5)	96.1 (85.4–100.0)	668.3 (481.5–927.8)	26.1 (17.9–38.2)	93.8 (81.8–99.3)	100.0 (90.8–100.0)	613.9 (425.9–884.9)	22.6 (14.9–34.3)	94.0 (82.5–99.3)	100.0 (91.1–100.0)
Seasonal H3N2	21.1 (15.2–29.3)	1.7 (1.4–2.1)	11.8 (4.9–24.6)	41.2 (27.9–56.0)	302.0 (179.0–509.7)	30.6 (18.5–50.7)	85.4 (71.6–94.1)	89.6 (76.6–96.8)	278.6 (190.2–408.1)	27.9 (18.5–42.0)	92.0 (79.9–98.2)	98.0 (88.0–100.0)
Seasonal B	37.9 (30.4–47.2)	1.1 (0.9–1.4)	9.8 (3.7–22.3)	68.6 (54.0–80.8)	151.0 (116.2–196.3)	4.1 (3.1–5.5)	66.7 (51.5–79.6)	97.9 (87.5–100.0)	141.2 (109.2–182.6)	4.5 (3.5–5.8)	64.0 (49.1–77.0)	94.1 (82.5–99.3)
Day 28			*N* = 51				*N* = 47					
Pandemic H1N1	403.2 (272.6–596.2)	44.3 (30.7–64.1)	96.1 (85.4–100.0)	98.0 (88.2–100.0)	70.1 (50.8–96.7)	8.7 (6.2–12.3)	70.2 (54.9–82.6)	78.7 (63.9–89.3)	–	–	–	–
Seasonal H1N1	230.9 (176.6–302.0)	7.5 (5.3–10.6)	78.4 (64.3–88.7)	100.0 (91.3–100.0)	505.5 (363.6–702.7)	19.4 (13.0–28.9)	91.5 (78.7–98.1)	100.0 (90.6–100.0)	–	–	–	–
Seasonal H3N2	132.3 (84.9–206.0)	10.6 (6.8–16.5)	76.5 (62.2–87.2)	84.3 (70.9–93.1)	272.1 (162.9–454.5)	27.2 (16.3–45.3)	83.0 (68.7–92.4)	87.2 (73.6–95.4)	–	–	–	–
Seasonal B	94.2 (72.4–122.5)	2.8 (2.1–3.7)	41.2 (27.9–56.0)	92.2 (80.3–98.2)	140.1 (103.9–188.9)	3.8 (2.8–5.3)	61.7 (46.4–75.5)	97.9 (87.3–100.0)	–	–	–	–
Day 42			*N* = 51				*N* = 46				*N* = 50	
Pandemic H1N1	622.8 (422.6–918.0)	68.5 (47.8–98.2)	96.1 (85.4–100.0)	98.0 (88.2–100.0)	296.8 (217.5–404.9)	36.3 (26.2–51.2)	100.0 (90.4–100.0)	100.0 (90.4–100.0)	220.1 (158.8–305.0)	30.7 (22.0–42.8)	94.1 (82.5–99.3)	94.0 (82.5–99.3)
Seasonal H1N1	606.1 (466.8–787.0)	19.6 (13.3–29.0)	92.2 (80.3–98.2)	100.0 (91.3–100.0)	466.4 (348.9–623.4)	17.5 (12.2–25.2)	93.5 (81.1–99.3)	100.0 (90.4–100.0)	541.9 (380.8–771.1)	20.0 (12.9–30.9)	92.0 (79.9–98.2)	100.0 (91.1–100.0)
Seasonal H3N2	342.5 (223.3–525.2)	27.6 (17.9–42.4)	92.2 (80.3–98.2)	96.1 (85.4–100.0)	263.1 (159.9–432.8)	26.3 (16.3–42.4)	84.8 (70.5–93.8)	89.1 (75.6–96.7)	239.2 (162.2–352.6)	23.9 (15.9–35.9)	86.0 (72.6–94.3)	94.0 (82.5–99.3)
Seasonal B	147.5 (117.6–184.9)	4.4 (3.4–5.6)	62.7 (48.1–75.8)	100.0 (91.3–100.0)	135.6 (107.2–171.4)	3.7 (2.8–4.8)	65.2 (49.7–78.6)	97.8 (87.0–100.0)	147.2 (119.0–182.2)	4.7 (3.7–6.1)	68.0 (53.2–80.4)	100.0 (91.1–100.0)

[a] GMT, geometric mean titres. Proportions of subjects are based on the total number of subjects tested at each time point. Seroconversion was defined as a titre before vaccination of less than 1:10 and a titre after vaccination of 1:40 or more, or a titre before vaccination of 1:10 or more and at least a fourfold increase after vaccination. Seropositive was defined as HI titre $\geq$ 1:40.

Table 2
Microneutralization antibody response against the pandemic H1N1 and seasonal H1N1 virus in three groups[a].

Day and immunogenecity	Pandemic H1N1			Seasonal H1N1		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Before vaccination	*N* = 51	*N* = 49	*N* = 51	*N* = 51	*N* = 49	*N* = 51
GMT	7.4	7.4	6.4	8.1	8.2	8.4
(95%CI)	(6.0–9.0)	(6.0–9.2)	(5.5–7.4)	(6.6–10.0)	(6.5–10.3)	(6.8–10.4)
Seropositivity	3.9	8.2	0.0	2.0	6.1	5.9
(95%CI)	(0.7–14.6)	(2.6–20.6)	(0.0–8.7)	(0.1–11.8)	(1.6–17.9)	(1.5–17.3)
Day 21	*N* = 51	*N* = 48	*N* = 50	*N* = 51	*N* = 48	*N* = 50
GMT	369.1	16.0	154.5	20.1	97.0	84.3
(95%CI)	(256.5–530.9)	(11.1–22.9)	(107.9–221.3)	(15.3–26.2)	(72.2–130.3)	(61.8–114.9)
GMT ratio	50.1	2.2	24.1	2.5	11.7	10.4
(95%CI)	(34.9–71.9)	(1.6–2.9)	(16.2–36.0)	(1.9–3.2)	(8.1–17.0)	(7.2–15.0)
Seroconversion	96.1	18.8	86.0	15.7	72.9	72.0
(95%CI)	(85.4–100.0)	(9.4–33.2)	(72.6–94.3)	(7.5–29.2)	(57.9–84.7)	(57.3–83.7)
Seropositivity	96.1	29.2	88.0	27.5	83.3	82.0
(95%CI)	(85.4–100.0)	(17.4–44.4)	(75.0–95.7)	(16.3–42.1)	(69.2–92.6)	(68.1–91.5)
Day 42	*N* = 51	*N* = 46	*N* = 50	*N* = 51	*N* = 46	*N* = 50
GMT	303.4	162.4	119.2	90.8	81.7	77.7
(95%CI)	(210.0–438.2)	(116.5–226.3)	(82.6–171.9)	(71.4–115.4)	(61.4–108.7)	(57.2–105.7)
GMT ratio	41.2	21.9	18.6	11.2	9.7	9.6
(95%CI)	(29.0–58.5)	(15.5–30.9)	(12.5–27.6)	(8.1–15.5)	(6.9–13.5)	(6.7–13.7)
Seroconversion	90.2	87.0	78.0	76.5	73.9	68.0
(95%CI)	(77.8–97.0)	(73.0–95.3)	(63.7–88.5)	(62.2–87.2)	(58.6–85.7)	(53.2–80.4)
Seropositivity	90.2	89.1	82.0	86.3	80.4	72.0
(95%CI)	(77.8–97.0)	(75.6–96.7)	(68.1–91.5)	(73.1–94.4)	(65.6–90.7)	(57.3–83.7)

[a] GMT, geometric mean titres. Proportions of subjects are based on the total number of subjects tested at each time point. Seroconversion was defined as a titre before vaccination of less than 1:10 and a titre after vaccination of 1:40 or more, or a titre before vaccination of 1:10 or more and at least a fourfold increase after vaccination. Seropositive was defined as HI titre ≥ 1:40.

or significant increases in GMT HI titre occurred in all 3 groups (94–100%) on day 42 (end-point of study).

In Group 1 21 days after immunization, pandemic H1N1 vaccine had induced HI titres against seasonal H1N1 with a GMT ratio of 3.9, a 49% seroconversion rate and a 96% seroprotection rate, but not against H3N2. Likewise, in Group 2, immunization with seasonal influenza vaccine could also significantly enhance the GMT ratio (5.3), seroconversion (52%) and seroprotection rates (63%) in HI titres against pandemic H1N1 virus. The pattern of antibody responses, as measured by the MN assay, was similar to those observed with the HI assay (Table 2).

At baseline antigen-specific antibody-secreting cells (ASC) for against all three influenza viruses were undetectable in all subjects (Fig. 2). After the first immunization antigen-specific ASC were detected in the periphery in all three groups, peaking seven days after immunization before returning to baseline level by day 21 post-immunization. After a second immunization, antigen-specific ASC were also detectable and peaked seven days after immunization before returning to baseline thereafter. In both Groups 1 and 2, immunization of pandemic H1N1 vaccine or seasonal influenza vaccine could induce elevation of heterologous antigen-specific ASC at a lower magnitude. The effect was more evident between pandemic H1N1 and seasonal H1N1 and less so on H3N2.

Although all three immunization strategies met European Union licensing criteria for influenza vaccines, the magnitude of the GMT for pandemic H1N1 was significantly reduced in Groups 2 and 3 21 days after immunization by both HI and MN assays (Fig. 3a and Fig. 1 in the Supplementary Appendix for Reverse Cumulative Curves). Prior immunization or co-immunization with seasonal influenza vaccine reduced the magnitude of GMT against pandemic H1N1 by more than 50% in Groups 2 ($p = 0.0046$) and 3 ($p = 0.0044$). A similar reduction in magnitude of GMT by MN assays was also observed (Fig. 3b). However, this effect on GMT was not observed against seasonal H1N1. The frequency of antigen-specific ASC to pandemic H1N1 was also diminished in Groups 2 and 3 but not against seasonal H1N1 (Fig. 3c).

4. Discussion

Our study demonstrates that administration of a pandemic H1N1 and a trivalent seasonal vaccine either sequentially or simultaneously is safe and immunogenic, inducing antibody responses that meet the criteria for licensing. However we observed differences in magnitude of the antibody responses that were dependent on the order of vaccination. Our results showed a significant reduction in the magnitude of the pandemic H1N1 antibody responses when the pandemic vaccine was administered alongside or after the seasonal flu vaccine when compared to vaccination with pandemic followed by seasonal vaccine. Antibody responses to seasonal H1N1 and seasonal H3N2 were not significantly different between the three groups indicating that the observed inhibitory effect is specific for seasonal vaccination on pandemic H1 responses and not vice versa.

Evidence that concomitant administration of a pandemic vaccine and a seasonal vaccine was immunogenic and well tolerated with mostly mild adverse reactions is in agreement with a study on simultaneous vaccination by Vajo et al. [6]. They found no significant differences in the pandemic antibody responses to pandemic vaccination alone and simultaneous pandemic and seasonal vaccination, although a decreasing trend in the GMT ratios was observed with the latter. The difference in observations may lie in their use of a lower dose plus adjuvant regime and/or the different vaccines used. By comparing simultaneous with a sequential vaccination schedule, as opposed to just pandemic vaccination alone, we have uncovered a quantitative difference in the levels of H1N1 antibody produced that may have important implications in the development of quadrivalent influenza vaccines for the future.

The nature and mechanism of this interference is not known and has not been explored in this paper but it brings to mind the concept of original antigenic sin (OAS). Proposed over half a century ago, OAS describes the phenomenon of infection with influenza inducing an antibody response against a previously experienced and closely related strain at the expense of spe-

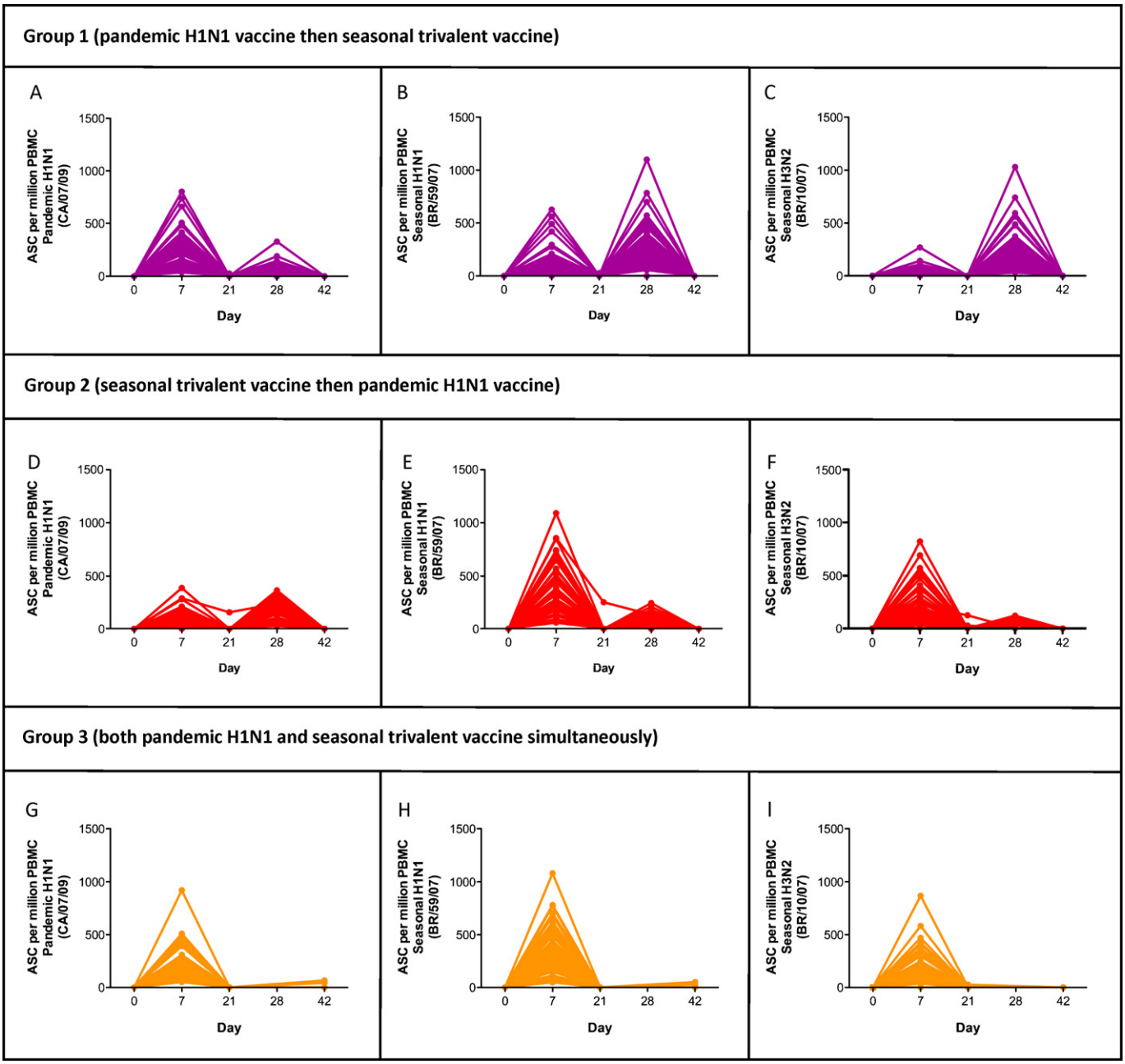

Fig. 2. Frequency of influenza-specific antibody secreting cells (ASC) in peripheral blood in study Groups 1 (Panels A–C), 2 (Panels D–F) and 3 (Panels G–I), as measured by B cell ELISPOT against indicated vaccine antigens from homologous X-179A strain (Panels A, D, G) and A/Brisbane/59/2007 H1N1-like (Panels B, E, H); A/Brisbane/10/2007 H3N2-like (Panels C, F, I). The data represented as the number of IgG-ASC/10^6 PBMC.

cific responses to the infecting strain [7,8]. In our study, despite observing lower titres of antibody to pandemic H1N1 after or alongside seasonal vaccination, a robust response did occur with pandemic H1N1 seropositivity rates comparable between all three groups. In addition in Group 1, no boosting effect on seasonal flu vaccine antibody responses by pandemic vaccination was seen; therefore the concept of OAS does not align with our observations. This agrees with a study in mice that described OAS in the context of live-virus infection but not with inactivated viruses [9].

An important question arising from our study is whether a fifty percent reduction in antibody titre as a result of seasonal vaccination interference has any impact on subsequent infection rates. Statistical modelling suggests that absolute antibody levels (mean and standard deviation of titres) as opposed to seropositivity rates are more closely related to clinical protection [10]. Thus although the 95%CI of the GMT for antibody against the pandemic virus is within the accepted protective range for all three vaccine regimens, vaccination strategy will depend on the availability of vaccine in relation to the perceived threat from circulating viruses and the vulnerability of different sections of the population. Since during the latter half of 2009 pandemic H1N1 was the predominant circulating virus in most parts of the world, as in China during October to December, a strategy of (initial) vaccination with the monovalent pandemic vaccine, as applied in China and many other countries, was most appropriate. Subsequent vaccination of the elderly and other 'at risk' groups with the trivalent seasonal vaccine would protect

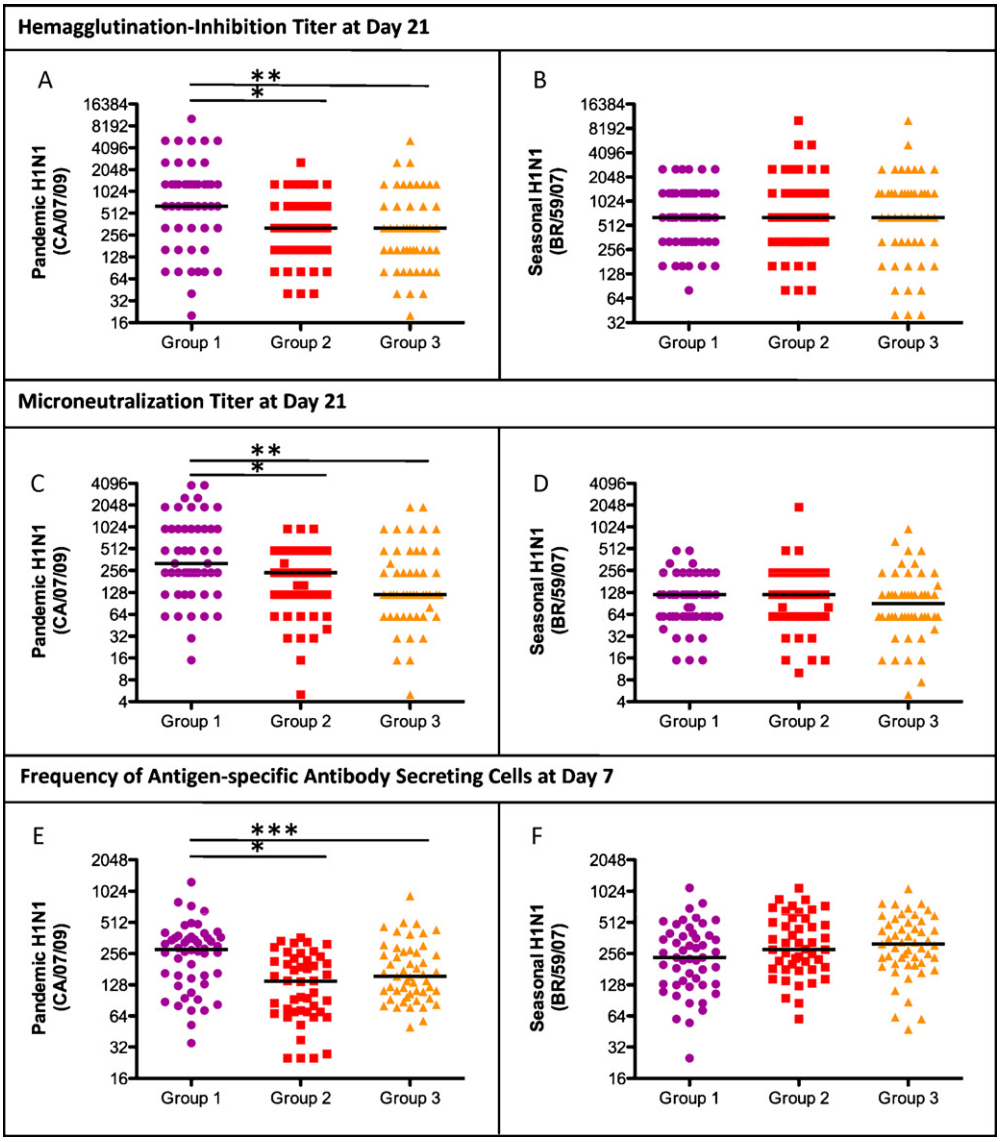

Fig. 3. Magnitudes of antibody response in three study groups 21 days after immunization of the vaccines as measured by haemagglutination-inhibition (HI) assay (Panels A and B), microneutralization (MN) assay (Panels C and D), and B cell ELISPOT (Panels E and F). Antibody titres specific to the pandemic H1N1 virus (Panels A, C, and E) or seasonal H1N1 virus (Panels B, D, and F) are expressed as reciprocal of the dilution and are given on a $\log_2$ scale. The bar indicates the median value. Differences between groups were tested using non-parametric Kruskal–Wallis's test with post hoc Dunn's test for multiple comparisons. A p value of less than 0.05 was considered significant ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.0001$).

against influenza B which was the predominant virus circulating in China during early 2010. This issue is no longer pertinent for current trivalent vaccines which contain the pandemic H1N1 virus. The impact on pandemic H1N1 infection levels will however only be apparent from future retrospective epidemiological studies.

Taken together, our data demonstrates that both sequential and simultaneous administration of pandemic H1N1 and seasonal trivalent vaccines induce satisfactory protective immune responses but individuals receiving pandemic H1N1 first have higher titres of protective antibody against pandemic H1N1 antigen. Therefore we would recommend initial immunization with the pandemic H1N1 monovalent vaccine followed by the seasonal trivalent vaccine, to maximise pandemic H1N1 antibody responses.

Conflicts of interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2010.11.058.

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