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Long-Term Immunogenicity of the Pandemic Influenza A/H1N1 2009 Vaccine among Health Care Workers: Influence of Prior Seasonal Influenza Vaccination

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Health care workers (HCWs) are at great risk of influenza infection and transmission. Vaccination for seasonal influenza is routinely recommended, but this strategy should be reconsidered in a pandemic situation. Between October 2009 and September 2010, a multicenter study was conducted to assess the long-term immunogenicity of the A/H1N1 2009 monovalent influenza vaccine among HCWs compared to non-health care workers (NHCWs). The influence of prior seasonal influenza vaccination was also assessed with respect to the immunogenicity of pandemic H1N1 influenza vaccine. Serum hemagglutinin inhibition titers were determined prevaccination and then at 1, 6, and 10 months after vaccination. Of the 360 enrolled HCW subjects, 289 participated in the study up to 10 months after H1N1 monovalent influenza vaccination, while 60 of 65 NHCW subjects were followed up. Seroprotection rates, seroconversion rates, and geometric mean titer (GMT) ratios fulfilled the European Union's licensure criteria for influenza A/California/7/2009 (H1N1) at 1 month after vaccination in both the HCWs and NHCWs, without any significant difference. At 6 months after vaccination, the seroprotection rate was more significantly lowered among the NHCWs than among the HCWs ($P < 0.01$). Overall, postvaccination (1, 6, and 10 months after vaccination) GMTs for A/California/7/2009 (H1N1) were significantly lower among the seasonal influenza vaccine recipients than among the nonrecipients ($P < 0.05$). In conclusion, HCWs should be encouraged to receive an annual influenza vaccination, considering the risk of repeated exposure. However, prior reception of seasonal influenza vaccine showed a negative influence on immunogenicity for the pandemic A/H1N1 2009 influenza vaccine.

Health care facilities can be a source for the rapid spread of influenza, and health care workers (HCWs) are considered the primary source of influenza transmission to their patients. Transmission has been shown to occur from patients to HCWs, from HCWs to patients, and among health care staff (1–4). Vaccines are the primary method of control for influenza and its complications. In fact, generalized vaccination of HCWs has been shown to have a positive impact on absenteeism rates and the economic burden associated with the seasonal epidemic (5). Nevertheless, based on the Advisory Committee on Immunization Practice (ACIP) recommendations, HCWs have one of the lowest influenza vaccine compliance rates (6–8).

During the 2009 influenza pandemic, HCWs were considered an important priority group for influenza vaccination, and it was recommended that they receive both the seasonal and the pandemic vaccines for fear of the emergence of a reassortant virus. However, it is unknown how such a vaccination strategy might affect the immunogenicity of a pandemic vaccine. Moreover, considering that influenza circulates longer during a pandemic (≥6 months), there was a concern that a single-dose influenza vaccine for HCWs would be insufficient to provide long-term protection.

In the present study, we evaluated the long-term immunogenicity of the A/H1N1 2009 influenza monovalent vaccine in HCWs aged 18 to 64 years. In addition, we evaluated the impact of prior seasonal influenza vaccination on the immunogenicity of the A/H1N1 2009 influenza monovalent vaccine.

MATERIALS AND METHODS

Study design. Between October 2009 and September 2010, we conducted a multicenter study to assess the immunogenicity of the A/H1N1 2009 monovalent influenza vaccine and its persistence after vaccination among subjects aged 18 to 64 years. The study was performed at four university hospitals in Korea. The primary objective of the study was to investigate both the short-term (1 month postvaccination) and the long-term (6 and 10 months postvaccination) immunogenicities of the influenza vaccine among HCWs compared to the general population (non-health care workers [NHCWs]). The immunogenicity of the A/H1N1 2009 monovalent influenza vaccine among HCWs was further analyzed according to whether or not they had received a seasonal influenza vaccine.

The exclusion criteria included a history of laboratory-confirmed infection with influenza A/H1N1 2009, prior receipt of an influenza A/H1N1 2009 monovalent vaccine, immunosuppression, hypersensitivity to any component of the vaccines (including eggs), history of Guillain-Barre syndrome, thrombocytopenia or any coagulation disorder contraindicating intramuscular injection, current febrile illness or another acute illness, administration of gamma globulin during the previous 3 months, and any other vaccination within the past 30 days.

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TABLE 1 Demographic characteristics of the study subjects[a]

Parameter	HCWs					
	Seasonal influenza vaccine recipients ($n = 209$)	Seasonal influenza vaccine nonrecipients ($n = 80$)	*P* value[b]	Total ($n = 289$)	NHCWs ($n = 60$)	*P* value[c]
Male sex, no. (%)	60 (28.7)	24 (30.0)	0.83	84 (29.1%)	13 (21.7%)	0.27
Mean age (yr) ± SD	34.7 ± 8.2	33.1 ± 8.9	0.16	34.2 ± 8.4	36.7 ± 10.2	0.07
Age group (yr), no. (%)			0.23			0.07
20–29	70 (33.5)	38 (47.5)		108 (34.9)	20 (33.3)	
30–39	80 (38.3)	23 (28.8)		103 (35.6)	14 (23.3)	
40–49	49 (23.4)	15 (18.8)		64 (22.1)	20 (33.3)	
50–59	9 (4.3)	3 (3.8)		12 (4.2)	6 (10.0)	
60–64	1 (0.5)	1 (0.1)		2 (0.7)	1 (1.7)	
Comorbidities, no.	2 (0.96)	1 (1.3)	0.83	3 (1.0)	1 (1.7)	0.54
Diabetes	1	1		2	1	
Chronic renal failure	0	0		0	0	
Liver cirrhosis	0	0		0	0	
Malignancy	1	0		1	0	
Immunosuppressant treatment	0	0		0	0	

[a] HCWs, health care workers; NHCWs, non-health care workers.
[b] Comparison between seasonal influenza vaccine recipients and nonrecipients.
[c] Comparison between HCWs and NHCWs.

The demographic data collected for the study subjects included age, gender, comorbidities, and history of vaccination for seasonal influenza (2009 to 2010). Each subject received one dose of 15 μg nonadjuvanted vaccine, which was administered intramuscularly into the deltoid muscle. On days 0, 30 ± 7, 180 ± 7, and 300 ± 7 postvaccination, a 10-ml venous blood sample was obtained from each subject.

The study was approved by the ethics committee of each institution involved and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. All subjects provided written informed consent before enrollment.

Vaccines. The influenza A/H1N1 2009 vaccine, a monovalent, nonadjuvanted, inactivated, split-virus vaccine (15 μg hemagglutinin antigen per 0.5-ml prefilled syringe), was produced by Green Cross Corporation (Yongin, South Korea). The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A, distributed by the National Institute for Biological Standards and Control in the United Kingdom. The vaccine was prepared in embryonated chicken eggs using standard techniques for the production of seasonal trivalent inactivated vaccines.

Immunogenicity assessment. Antibody responses were detected by means of hemagglutination inhibition (HI) assays, according to established procedures and with use of turkey erythrocytes (9, 10), at the Korea University Guro Hospital. Titers of antihemagglutinin (anti-HA) antibodies that were below the detection limit (i.e., <1:10) were assigned a value of 1:5, and titers above 1:5,120 were assigned a value of 1:5,120.

The serologic response, measured by HI antibody titer, was assessed using the European Agency for the Evaluation of Medicinal Products (EMA) criteria as follows: seroprotection rate (the percentage of subjects with a postvaccination titer of ≥1:40), seroconversion rate (either a postvaccination titer of ≥1:40 in subjects with a prevaccination titer of <1:10 or a ≥4-fold titer increase in subjects with a prevaccination titer of ≥1:10), and geometric mean titer (GMT) fold change (GMT ratio of postvaccination titer to prevaccination titer) (11). The EMA definition of seroprotection was used at 1, 6, and 10 months after vaccination to directly compare the immunologic persistence values among the three postvaccination time points. All of the following criteria had to be met to confirm protective immunogenicity: seroprotection rate of >70%, seroconversion rate of >40%, and GMT fold change of >2.5.

Statistical analysis. All statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL). Descriptive statistics are reported as the number of subjects and the corresponding percentage. HI antibody titers are expressed as geometric mean with a 95% confidence interval. Seroprotection and seroconversion rates were compared using the chi-square test, while comparison of GMTs and their fold changes was conducted with Student's *t* test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics of study subjects. Of the 360 enrolled HCWs, 289 completed the study up to 10 months after the initial A/H1N1 2009 monovalent influenza vaccination. Among the 79 subjects who dropped out, two were diagnosed with influenza A/H1N1 2009 during the follow-up period, and the others refused follow-up after the initial consent. Sixty of the 65 NHCW subjects were followed up until 10 months postvaccination; the five subjects who dropped out refused follow-up after the initial consent. Among the 289 HCWs, 209 received a seasonal influenza vaccine at least 3 weeks before A/H1N1 2009 vaccination. The baseline characteristics of the study subjects are presented in Table 1.

Immunogenicity of 2009 pandemic influenza vaccine: health care workers versus non-health care workers. Seroprotection rates, seroconversion rates, and GMT fold changes fulfilled the EMA criteria for influenza A/California/7/2009 (H1N1) at 1 month after vaccination in both the HCWs and the NHCWs, without a significant difference (Table 2). Irrespective of previous vaccination for seasonal influenza ($P = 0.51$), rates of seroprotection and seroconversion met the EMA criteria in the HCWs. However, GMTs for A/California/7/2009 (H1N1) were significantly lower among the seasonal influenza vaccine recipients than among the nonrecipients at 1 month postvaccination ($P = 0.01$).

Immunogenicity of 2009 pandemic influenza vaccine against 2009-2010 seasonal influenza vaccine strain. We assessed HI titers against the 2009-2010 seasonal influenza A/H1N1 vaccine strain (influenza A/Brisbane/59/2007) with baseline and postvaccination (at 1 month) samples after the pandemic influenza vaccination. GMTs for influenza A/Brisbane/59/2007 (H1N1) did not increase remarkably

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TABLE 2 Comparison of short and long-term immunity values for influenza A/H1N1 2009 for seasonal influenza vaccine recipients versus nonrecipients[a]

Immunogenicity end point	HCWs					
	Seasonal influenza vaccine recipients ($n = 209$)	Seasonal influenza vaccine nonrecipients ($n = 80$)	*P* value[b]	Total ($n = 289$)	NHCWs ($n = 60$)	*P* value[c]
Seroprotection rate, % (95% CI)						
1 mo postvaccination	89.5 (84.6–92.9)	92.5 (84.6–96.5)	0.51	90.3 (86.3–93.2)	85.0 (73.8–91.8)	0.25
6 mo postvaccination	90.4 (85.7–93.7)	91.3 (83.0–95.6)	0.83	90.7 (86.7–93.5)	66.7 (54.0–77.3)	<0.01
10 mo postvaccination	53.1 (46.3–59.8)	68.8 (57.9–77.8)	0.02	57.4 (51.7–63.0)	61.7 (49.0–72.9)	0.57
Seroconversion rate, % (95% CI)						
1 mo postvaccination	81.8 (76.0–86.4)	81.3 (71.3–88.3)	0.91	79.9 (74.9–84.1)	76.7 (64.5–85.5)	0.37
6 mo postvaccination	86.6 (81.3–90.6)	87.5 (78.5–93.0)	0.84	84.0 (79.4–87.8)	50.0 (37.7–62.3)	<0.01
10 mo postvaccination	45.0 (38.4–51.8)	55.0 (44.1–65.4)	0.13	43.6 (38.0–49.4)	46.7 (34.6–59.2)	0.54
GMT (95% CI)						
Prevaccination	12.7 (11.5–13.9)	14.5 (188.0–271.3)	0.16	13.1 (12.1–14.3)	10.5 (8.1–13.6)	0.10
1 mo postvaccination	110.7 (94.4–129.9)	167.1 (124.2–224.9)	0.01	124.1 (107.6–143.1)	146.1 (97.7–218.6)	0.45
6 mo postvaccination	93.9 (81.8–107.7)	167.1 (127.8–218.5)	<.01	110.1 (97.0–125.0)	47.4 (33.7–66.7)	<0.01
10 mo postvaccination	31.7 (26.7–37.6)	55.6 (39.9–77.6)	<0.01	37.0 (31.7–43.3)	40.9 (29.9–55.9)	0.60
GMT ratio (95% CI)						
1 mo postvaccination	8.7 (7.4–10.3)	11.5 (8.4–15.8)	0.13	9.4 (8.1–10.9)	13.9 (9.1–21.4)	0.09
6 mo postvaccination	7.4 (6.5–8.5)	11.5 (8.7–15.2)	<0.01	8.4 (7.4–9.5)	4.5 (3.2–6.4)	<0.01
10 mo postvaccination	2.5 (2.1–3.0)	3.8 (2.7–5.4)	0.03	2.8 (2.4–3.3)	3.8 (2.8–5.3)	0.10

[a] Geometric mean titer ratios are the ratios of the antibody level at the day of interest compared to that at day 0. Seroconversion was defined as a prevaccination antibody titer of ≤1:10 and a postvaccination titer of ≥1:40. HI, hemagglutination inhibition; CI, confidence interval; GMT, geometric mean titer; HCWs, health care workers; NHCWs, non-health care workers.
[b] Comparison between seasonal influenza vaccine recipients and nonrecipients.
[c] Comparison between HCWs and NHCWs.

in the seasonal vaccine recipients at 1 month after the pandemic influenza vaccination (GMT fold change, 1.1) (Table 3).

Immunologic persistence of 2009 pandemic influenza vaccine: health care workers versus non-health care workers. Prevaccination GMTs at and GMTs at 1 month postvaccination were indistinguishable between the HCWs and the NHCWs, whereas GMTs at 6 and 10 months postvaccination were significantly higher in the HCWs than in the NHCWs ($P < 0.01$). At 6 months after vaccination, the seroprotection rate was more significantly lowered among the NHCWs than among the HCWs ($P < 0.01$). As for the HCWs, all three EMA criteria were fully met even at 6 months after vaccination. Seroprotection was preserved in more than half of both the HCWs and the NHCWs (57.4% versus 61.7%; $P = 0.57$) up to 10 months postvaccination.

As for the vaccination status for seasonal influenza, overall postvaccination (1, 6, and 10 months after vaccination) GMTs for A/California/7/2009 (H1N1) were significantly lower among the seasonal influenza vaccine recipients than among the nonrecipients (1 month, $P = 0.01$; 6 and 10 months, $P < 0.01$) (Table 2). Though the difference was insignificant up to 6 months after vaccination, there was a significant difference in the seroprotection rates for A/California/7/2009 (H1N1) according to the vaccination status for seasonal influenza at 10 months postvaccination (recipients, 53.1%; nonrecipients, 68.8% [$P = 0.02$]).

TABLE 3 Comparison of immunity against A/Brisbane/59/2007 (H1N1) for the seasonal influenza vaccine recipients and nonrecipients pre- and postvaccination at 1 month after influenza A/H1N1 2009 vaccination[a]

Parameter	Seasonal influenza vaccine recipients ($n = 209$)	Seasonal influenza vaccine nonrecipients ($n = 80$)	*P* value
GMT (95% CI)			
Prevaccination	62.3 (54.4–71.3)	37.9 (27.5–52.1)	<.01
Postvaccination	68.6 (62.3–75.5)	78.6 (64.2–96.1)	0.23
GMT ratio (95% CI)	1.1 (1.0–1.1)	2.1 (1.8–2.3)	0.01

[a] A/Brisbane/59/2007 (H1N1) is the 2009-2010 seasonal influenza vaccine strain. GMT, geometric mean titer; CI, confidence interval.

DISCUSSION

This study shows that the 2009 pandemic H1N1 vaccine can induce long-term immunity as assessed by serological assays, which is in line with several previous reports (12–14). Vaccination is the primary tool for the control of influenza. According to a previous study, the overall vaccine effectiveness for influenza A/California/7/2009 (H1N1) was 73.4% in Korea (15). Based on the results of the present study, in accordance with ACIP recommendations, all HCWs should be encouraged to receive an influenza vaccination. During the 2009 pandemic, the A/H1N1 2009 vaccine coverage rate in HCWs was reported to be greater than 90% in Korea, which was remarkably higher than those during interpandemic periods (16).

Another important finding of this study is the negative effect of prior seasonal influenza vaccination on the immunogenicity of the A/H1N1 2009 pandemic vaccine. A reduced HI antibody response against A/California/7/2009 (H1N1) was noted in healthy adults who had previously received a seasonal influenza vaccine. This finding has been presented before in the ferret model (17) as well as in clinical studies (18, 19). The mechanism is still uncertain, but the following hypothesis may be considered. There is a chance that a recent exposure to a seasonal vaccine may hamper

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the production of new antibodies by the pandemic influenza vaccine, which preferentially reactivates previously activated high-affinity memory B cells rather than naive B cells (the hypothesis of original antigenic sin) (20). Contrary to our expectation, GMTs for the 2009-2010 seasonal influenza A/H1N1 vaccine strain (A/Brisbane/59/2007) did not increase remarkably in the seasonal vaccine recipients at 1 month after the pandemic influenza vaccination. Another possibility with respect to original antigenic sin is the generation of antibodies of lower affinity against pandemic influenza virus. Allowing for these points, in a pandemic situation with a new influenza virus, routine generalized vaccination for seasonal influenza needs to be reconsidered.

Interestingly however, a recent study with ferrets showed that prior seasonal influenza vaccination induced a positive immunologic priming effect on subsequent MF59-adjuvanted A/H1N1 2009 influenza vaccination (21). MF59 adjuvant might enhance the activity of CD4 T cells and memory B cells. Moreover, in the study by Langley et al. (22), subjects immunized first with seasonal influenza vaccine and then with two doses of $AS0_3$-adjuvanted pandemic influenza A/H1N1 2009 vaccine had noninferior immune responses to the pandemic strain compared to those subjects receiving two doses of $AS0_3$-adjuvanted pandemic influenza A/H1N1 2009 vaccine without previous receipt of seasonal vaccine. Further studies are required to better clarify whether new adjuvants (MF59 and $AS0_3$) may overcome the negative impact from prior seasonal influenza vaccination or not.

In this study, the seroprotection rate was more significantly lowered among the NHCWs than among the HCWs at 6 months after vaccination. This might reflect repeated exposure of HCWs to influenza virus without disease development, although only two (0.6%) of the 360 HCWs had laboratory-confirmed influenza A/California/7/2009 (H1N1) infection.

Our study has some limitations. First, the degree of exposure to influenza in each HCW was not assessed. Second, data on influenza vaccination and infection in the previous year were not collected.

In summary, HCWs should be encouraged to receive annual influenza vaccinations. However, receipt of seasonal influenza vaccine showed a negative influence on the immunogenicity of pandemic influenza A/H1N1 2009 vaccine during the 2009 and 2010 seasons.

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We have no conflict of interest to declare.

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