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Comparison of the Long-Term Immunogenicity of Two Pandemic Influenza A/H1N1 2009 Vaccines, the MF59-Adjuvanted and Unadjuvanted Vaccines, in Adults

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Since the first reports of the A/H1N1 virus in April 2009, the pandemic influenza virus spread globally and circulated for a long time. The primary method for the control of influenza is vaccination, but levels of influenza vaccine-induced antibody are known to decline rapidly during a 6-month period. In adults aged 18 to 64 years, we compared the long-term immunogenicity of two of the influenza A/H1N1 2009 monovalent vaccines, 3.75-μg MF59-adjuvanted vaccine and 15-μg unadjuvanted vaccine. The serum hemagglutinin inhibition (HI) titers were determined prevaccination and at 1, 6, and 10 months after vaccination. One hundred six (88.3%) of the 120 subjects were monitored for the entire 10-month period after receiving the influenza A/H1N1 2009 monovalent vaccine. There were 60 patients who received the unadjuvanted vaccine and 46 patients who received the MF59-adjuvanted vaccine. The seroprotection rates, seroconversion rates, and the geometric mean titer (GMT) folds fulfilled the criteria of the European Medicines Agency (EMA) for influenza A/California/7/2009 (H1N1) at 1 month after vaccination irrespective of the vaccine composition. Although the GMTs at 1 month postvaccination were somewhat higher in the unadjuvanted vaccine recipients than in the MF59-adjuvanted vaccine recipients, the difference was not significant ($P = 0.29$). The seroprotection rates at 6 and 10 months postvaccination were preserved above 70% but only in the MF59-adjuvanted vaccine recipients. In conclusion, low-dose MF59-adjuvanted influenza vaccine, even with 3.75 μg hemagglutinin antigen, might induce excellent long-term immunity that is comparable to the conventional dose of unadjuvanted vaccine among healthy adults aged 18 to 64 years.

The pandemic influenza A/H1N1 virus, first reported in April 2009, spread globally and circulated for a year. Although it is accepted that pandemic influenza vaccines play an essential role in the control of influenza, we wondered whether it would be effective for a long period during the second or third wave of the pandemic. Furthermore, we do not know the pandemic vaccine's immunogenicity against potentially more virulent mutant viruses.

A high-dose vaccine, intradermal delivery system, and many adjuvants have been used to achieve a strong immune response after vaccination. Among them, vaccine adjuvant is known to elicit a strong, broad immune response and induce long-term protection against infectious diseases. Contrary to other adjuvants, MF59 (oil-in-water emulsions) does not induce a depot effect (a delayed release of antigen over time). However, MF59 directly enhances antigen uptake by activated dendritic cells, induces chemokine production, and also is involved in the recruitment of cells to the tissues (5, 12).

During the 2009 to 2010 influenza pandemic in the Republic of Korea, doses containing 15 μg of unadjuvanted 2009 A/H1N1 monovalent influenza vaccine were produced initially, but the 3.75-μg MF59-adjuvanted vaccine (used as an antigen-sparing strategy) was mainly distributed later. In the present study, we evaluated the long-term immunogenicity of the two kinds of 2009 A/H1N1 influenza monovalent vaccines (unadjuvanted vaccine versus MF59-adjuvanted vaccine) in adults aged 18 to 64 years.

MATERIALS AND METHODS

Study design. Between October 2009 and September 2010, we conducted an observational, open-label, multicenter study to assess the immunogenicity of the influenza A/H1N1 2009 monovalent vaccine and the persistence of antibody response after vaccination in adults aged 18 to 64 years. The study was performed at three university hospitals located in southwestern Seoul, South Korea. The primary objective of the study was to investigate the immunogenicity of the influenza A/H1N1 2009 monovalent vaccine during the short term (1 month postvaccination) and long term (6 and 10 months postvaccination). We also compared the immunogenicity based on the vaccine formulation. Initially, 120 subjects who had been recruited for the study were divided into two groups: the unadjuvanted vaccine recipients (65 subjects) and the MF59-adjuvanted vaccine recipients (55 subjects). The secondary objective of the study was to assess the immunogenicity of the 2009 A/H1N1 monovalent influenza vaccine against the D222G mutant virus.

The exclusion criteria included a history of laboratory-confirmed infection with influenza A/H1N1 2009 or a history of an influenza A/H1N1 2009 monovalent vaccination. Patients who used immunosuppressants, had a hypersensitivity to any component of the vaccines (including eggs), or had a history of Guillain-Barre syndrome were also excluded. Other exclusion criteria included thrombocytopenia or any coagulation disorder contraindicating intramuscular injection, current febrile illness, or another acute illness. Finally, any patient who was administered gamma

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TABLE 1 Demographic characteristics of the study subjects

Characteristic	Unadjuvanted vaccine recipients ($n = 60$)	MF59 adjuvanted vaccine recipients ($n = 46$)	*P* value
Male sex, no. (%)	13 (21.7)	15 (32.6)	0.21
Age (yr), means ± SD	36.7 ± 10.2	36.3 ± 12.9	0.70
Comorbidity (%)	1 (1.7)	1 (1.3)	0.85
Diabetes	1	1	
Chronic renal diseases	0	0	
Liver cirrhosis	0	0	
Malignancy	0	0	

globulin during the previous 3 months or any other vaccination within the past 30 days was excluded.

The demographic data for the study subjects included age, sex, and comorbidities. Each subject received one dose administered intramuscularly into the deltoid muscle of either the 15-μg unadjuvanted vaccine or the 3.75-μg MF59-adjuvanted vaccine. Venous blood samples of 10 ml were collected from each subject on day 0 as well as 30 ± 7, 180 ± 7, and 300 ± 7 days after vaccination. 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 Practice. All subjects provided written, informed consent before enrollment.

Vaccines. The influenza A (H1N1) vaccine was obtained from the Green Cross Corporation (Yongin, South Korea). The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A that was 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 vaccine.

In this study, the unadjuvanted influenza vaccine was a split-virus product of 15 μg hemagglutinin antigen per 0.5-ml prefilled syringe. The MF59-adjuvanted vaccine was prepared by mixing the same split-virus product of 3.75 μg hemagglutinin antigen and 4.875 mg MF59C.1 (Novartis, Marburg, Germany) in a 0.125-ml dose. MF59C.1 consists of the following: squalene, polysorbate 80, sorbitan trioleate, trisodium citrate dehydrate, citric acid monohydrate, and water for injection (5, 12).

Immunogenicity assessment. The hemagglutination-inhibiting (HI) antibodies for the A/California/7/2009 (H1N1) virus and the D222G mutant virus were measured using a standard microtiter assay according to established procedures and with the use of turkey erythrocytes (8, 9). The D222G mutant virus was obtained by reverse genetic engineering. Titers of 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 the HI antibody titer, was assessed using the following criteria of the European Agency for the Evaluation of Medicinal Products (EMA): 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, GMT ratio of the postvaccination titer to prevaccination titer (6). The EMA definition of seroprotection was used at 1, 6, and 10 months after vaccination to directly compare the immunologic persistence among the three postvaccination time points. All of the following criteria must be met to confirm protective immunogenicity: a seroprotection rate of >70%, a seroconversion rate of >40%, and a GMT fold of >2.5.

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

TABLE 2 Short- and long-term immune responses after influenza A/H1N1 2009 vaccination, as measured by the HI assay[a]

Criterion	Immune response of: Unadjuvanted vaccine recipients ($n = 60$)	MF59 adjuvanted vaccine recipients ($n = 46$)	*P* value
Seroprotection rate, %			
1 mo postvaccination	85.0 (73.8–91.8)	80.4 (66.7–89.3)	0.61
6 mo postvaccination	66.7 (54.0–77.3)	73.9 (59.7-84.4)	0.52
10 mo postvaccination	61.7 (49.0–72.9)	71.7 (57.4-82.7)	0.31
Seroconversion rate, %			
1 mo postvaccination	76.7 (64.5–85.5)	71.7 (57.4–82.7)	0.85
6 mo postvaccination	50.0 (37.7–62.3)	58.7 (44.3–71.7)	0.24
10 mo postvaccination	46.7 (34.6–59.2)	56.5 (42.2–69.8)	0.33
GMT			
Prevaccination	10.5 (8.1–13.6)	13.7 (10.4–18.1)	0.18
1 mo postvaccination	146.1 (97.7–218.6)	109.8 (74.7–161.3)	0.29
6 mo postvaccination	47.4 (33.7–66.7)	56.6 (38.5–83.1)	0.56
10 mo postvaccination	40.9 (29.9–55.9)	50.1 (33.8–74.5)	0.41
GMT (fold[b])			
1 mo postvaccination	13.9 (9.1–21.4)	8.0 (5.2–12.3)	0.09
6 mo postvaccination	4.5 (3.2–6.4)	4.1 (2.7–6.2)	0.69
10 mo postvaccination	3.8 (2.8–5.3)	3.7 (2.4–5.6)	0.85

[a] Values in parentheses are 95% CIs.
[b] The GMT fold is the ratio of the antibody level at the day of interest to that on day 0. Seroconversion was defined as a prevaccination antibody titer of ≤1:10 and a postvaccination titer of ≥1:40.

RESULTS

Study subjects. One hundred six (88.3%) of the 120 subjects were monitored for the entire 10-month period after receiving the H1N1 monovalent influenza vaccination. The patients were divided into two groups: the unadjuvanted vaccine recipients (60 subjects) and the MF59-adjuvanted vaccine recipients (46 subjects). The 14 subjects that dropped out refused to follow up after providing initial consent. No subject was diagnosed with influenza A/H1N1 during follow-up. The demographic and baseline characteristics of the study subjects are presented in Table 1.

Immunogenicity and immunologic persistence. The seroprotection rates, seroconversion rates, and GMT folds fulfilled the EMA criteria for influenza A/California/7/2009 (H1N1) at 1 month after vaccination irrespective of vaccine composition (Table 2). However, the GMTs at 1 month postvaccination were higher in the unadjuvanted vaccine recipients than in the MF59-adjuvanted vaccine recipients. These findings were without statistical significance ($P = 0.29$). The seroprotection rates at 6 and 10 months postvaccination were preserved above 70% only in MF59-adjuvanted vaccine recipients (Table 2). The seroconversion rate met EMA criteria even at 10 months postvaccination irrespective of vaccine composition.

Immunogenicity against the D222G mutant virus. The immunogenicity against the D222G mutant virus was assessed in the 60 unadjuvanted vaccine recipients and the 46 MF59-adjuvanted vaccine recipients prevaccination and at 1 month after vaccination

TABLE 3 Comparison of immunogenicity against D222G mutant strain: unadjuvanted vaccine recipients versus MF59-adjuvanted vaccine recipients[a]

Criterion and virus type	Immune response of:		
	Unadjuvanted vaccine recipients ($n = 60$)	MF59-adjuvanted vaccine recipients ($n = 46$)	*P* value
Wild-type virus			
Seroprotection rate, %	85.0 (73.8–91.8)	80.4 (66.7–89.3)	0.61
Seroconversion rate, %	76.7 (64.5–85.5)	71.7 (57.4–82.7)	0.85
GMT fold	13.9 (9.1–21.4)	8.0 (5.2–12.3)	0.09
D222G mutant virus			
Seroprotection rate, %	71.7 (59.2–81.5)	69.6 (55.1–80.9)	0.83
Seroconversion rate, %	63.3 (50.6–74.4)	63.0 (48.5–75.5)	0.98
GMT fold	9.6 (6.2–14.7)	6.7 (4.4–10.1)	0.23

[a] Values in parentheses are 95% CIs.

(Table 3). Compared to the vaccine antibody response against wild-type virus, the rates of seroprotection and seroconversion were decreased ≥10% in both the unadjuvanted vaccine and the MF59-adjuvanted vaccine recipients, but these were still remarkably high. There was no statistically significant difference in the seroprotection rates (71.7 versus 69.6%; $P = 0.83$), seroconversion rates (63.3 versus 63.0%; $P = 0.98$), or the GMT folds (9.6 versus 6.7; $P = 0.23$) for the D222G mutant virus between the unadjuvanted vaccine and the MF59-adjuvanted vaccine recipients.

DISCUSSION

To the best of our knowledge, this report is the first to suggest that the MF59-adjuvanted influenza vaccine, even with 3.75 μg hemagglutinin antigen, induces excellent long-term immunogenicity for up to 10 months. Furthermore, the MF59-adjuvanted pandemic vaccine (A/H1N1 2009) used in South Korea was a bedside mixture of 3.75 μg of HA containing split vaccine and a half dose of MF59. We postulate that the immunogenicity of split-type influenza vaccine is potentiated even with low-dose MF59 adjuvant as well. Likewise, Ferguson et al. reported that a single dose of the 3.75-μg HA, $AS03_A$-adjuvanted H1N1 2009 influenza vaccine was highly immunogenic in adults until at least 6 months after single-dose vaccination. The immunogenicity of the 3.75-μg HA, $AS03_A$-adjuvanted vaccine was not inferior to that of 7.5- to 15-μg unadjuvanted vaccine (7).

The mechanism underlying the excellent long-term immunogenicity of the MF59-adjuvanted vaccine is uncertain. It appears that the MF59-adjuvanted vaccines can more effectively activate naive B cells with new specificities and reshape the preexisting memory of B-cell specificity. The MF59 vaccines induce strong $CD4^+$ T-cell help and more germinal center reactions, thereby producing long-lasting, high-quality antibodies (4, 12). Of note, the GMT and seroprotection rate decreased steadily in the MF59-adjuvanted vaccine recipients during the 10-month period.

D222G mutant virus is known to have higher virulence by increased α-2,3 sialic acid receptor preference compared to that of the parent strain (13). In this study, both the MF59-adjuvanted and unadjuvanted vaccines showed considerable immunogenicity against the D222G mutant virus. The immunogenicity was not enhanced remarkably with MF59 adjuvant. Many studies have previously shown that the MF59-adjuvanted influenza vaccine confers enhanced immunogenicity against heterovariant viral strains (1, 3, 11). Theoretically, MF59 might directly affect the quality of the immune response by inducing antibodies against epitopes in the vaccine that would not otherwise have been induced (4, 12). According to a study by Khurana et al., MF59 induced the spread of the epitope from HA2 to HA1, by which a much greater breadth of antigens participate in the immune response (10). In contrast, the sera from subjects vaccinated with the unadjuvanted or with the alum-adjuvanted vaccines mostly recognized the fragments of the HA stem region, which is localized to the HA2 region. In this study, however, the MF59 adjuvant-related difference was not observed for the cross-reactive immunogenicity. The antigenic variation of D222G might not be enough to make a difference according to the vaccine composition. In addition, there is a chance that the content (amount and ratio) of hemagglutinin antigen and MF59 adjuvant were not optimal. Based on the results of the clinical trials, the lowest concentration of the MF59-adjuvanted vaccine (3.75 μg hemagglutinin antigen and 4.875 mg MF59) was selected as an antigen-sparing strategy in the Republic of Korea during the 2009 and 2010 pandemic seasons (2).

Although this study examined only monovalent pandemic influenza A/H1N1 vaccine, increased immunogenicity has been reported with MF59 adjuvant in seasonal trivalent influenza vaccines and less immunogenic H5N1 vaccines (12). However, the evaluation of the long-term immunogenicity of these vaccines is also warranted, considering the potential use of MF59 in an interpandemic period and possible future pandemic situation by avian influenza. Given the reported effects of MF59 adjuvant on altering the focus of humoral responses from HA2 to HA1, further studies are required to better clarify immunogenic differences regarding MF59 adjuvant with alternative methods, including the microneutralization test and neuraminidase inhibition assay.

In summary, the low-dose MF59-adjuvanted influenza vaccine, even with 3.75 μg hemagglutinin antigen, might induce excellent long-term immunity comparably to the conventional-dose unadjuvanted vaccine among healthy adults aged 18 to 64 years. The immunogenicity against D222G mutant virus was remarkable irrespective of the MF59 adjuvant used.

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The authors have no conflicts of interest to declare.

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