Immunogenicity and Safety of the Influenza A/H1N1 2009 Inactivated Split-Virus Vaccine in Young and Older Adults: MF59-Adjuvanted Vaccine versus Nonadjuvanted Vaccine

Hee Jin Cheong,1 Joon Young Song,1 Jung Yeon Heo,1 Ji Yun Noh,1 Won Suk Choi,1 Dae Won Park,1 Seong-Heon Wie,2 and Woo Joo Kim1*

Division of Infectious Diseases, Department of Internal Medicine, Korea University College of Medicine,1 and Catholic University College of Medicine,2 Seoul, South Korea

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Since initial reports in April 2009, the pandemic influenza A (H1N1) virus has spread globally. Influenza vaccines are the primary method for the control of influenza and its complications. We conducted a multicenter clinical trial to evaluate the immunogenicity and safety of H1N1 vaccine (Green Cross Co.) in young adults (18 to 64 years) and the elderly (≥65 years) using a two-dose regimen, with the doses administered 21 days apart. Three different regimens of hemagglutinin antigen were comparatively analyzed: 3.75 μg (MF59 adjuvanted) versus 7.5 μg (MF59 adjuvanted) versus 15 μg (nonadjuvanted) in young adults and 3.75 μg (MF59 adjuvanted) versus 7.5 μg (MF59 adjuvanted) in the elderly. In young adults, all three vaccine regimens met the European Agency for the Evaluation of Medicinal Products (EMA) criteria after the first dose. In the elderly, on day 21 after the first dose, the rates of seroprotection and seroconversion were significantly higher for the 7.5-μg dose of MF59 adjuvanted vaccine than for the 3.75-μg dose (58.0% versus 44.3% [P = 0.03] and 53.7% versus 37.2% [P < 0.01], respectively). After the second dose, the geometric mean titer (GMT) increment was blunted with a 15-μg dose of nonadjuvanted vaccine, whereas the GMT increased about 2-fold with MF59 adjuvanted vaccines. In conclusion, a single 7.5-μg dose of MF59 adjuvanted vaccine would have a practical advantage over a two-dose, 3.75-μg, MF59 adjuvanted vaccine priming schedule. Following a two-dose priming schedule, the increase in hemagglutinin inhibition titers was higher with MF59 adjuvanted vaccine than with nonadjuvanted vaccine.

Since first reports in April 2009, the influenza A (H1N1) virus has spread globally (1). Korean influenza sentinel surveillance (KISS) reported that weekly influenza-like-illness (ILI) rates had already exceeded the seasonal outbreak criteria (2.6 per 1,000 cases) in week 34 and were about 10-fold higher than the recent seasonal average observed between October and December 2008 (14).

Influenza vaccines are the primary method of control for influenza and its complications. Seasonal influenza vaccines are unlikely to provide substantial cross-protection against pandemic H1N1 virus (9); therefore, influenza vaccine manufacturers accelerated the development of 2009 pandemic H1N1 influenza vaccine. In the Republic of Korea, pandemic influenza vaccine was produced by Green Cross Corporation (Yongin, South Korea) using the same procedures that have been used for the production of the company’s seasonal trivalent inactivated vaccine. We conducted a clinical trial with healthy young adults aged 18 to 64 years and with elderly adults aged ≥65 years to examine the immunogenicity and safety of this monovalent split-virus influenza A (H1N1) vaccine.

Concerning the vaccine shortage, adjuvanted influenza vac-
cines have been developed as a dose-sparing strategy. Adju-
vants might enhance the immunogenicity of influenza vaccines through several mechanisms, including triggering the release of chemokines, stimulating the maturation of resident dendritic cells, stimulating endocytosis, and/or enhancing the mig-
ration of the mature dendritic cells to the draining lymph nodes (25). Currently, several adjuvanted influenza vaccines are available: MF59 (Norvatis Vaccine and Diagnostics), ASO3 (GlaxoSmithKline [GSK]), AFO3 (Sanofi Pasteur), etc. (25). According to a recent report, a single dose of 3.75 μg of MF59 adjuvanted H1N1 influenza vaccine generated a favor-
able antibody response in healthy adults (aged 18 to 50 years) within 21 days after vaccination (5).

In this study, we comparatively analyzed three different regi-
imens of hemagglutinin antigen: 3.75 μg MF59 adjuvanted, 7.5 μg MF59 adjuvanted, or 15 μg nonadjuvanted. For each regi-
imen, a two-dose priming regimen was used in view of the low level of preexisting immunity.

MATERIALS AND METHODS

Study design. From September 2009 through November 2009, we conducted a multicenter, open-label clinical trial (clinical trial no. NCT01201902) at three sites in Seoul and Gyeonggi province, Republic of Korea: Korea University Guro Hospital, Korea University Ansan Hospital, and Catholic University St. Vin-
cent’s Hospital. The study was sponsored by Green Cross Corporation. The purpose of this study was to evaluate the immunogenicity and safety of H1N1 vaccine in healthy adults using a two-dose regimen, with the doses administered 21 days apart. We enrolled healthy young adults aged 18 to 64 years (group 1) and elderly individuals aged ≥65 years (healthy and living independently; group 2). The exclusion criteria included the following: a history of laboratory-con-
firmed 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-Barré 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 receipt of licensed inactivated or live vaccines within the preceding 4 weeks.

Subjects were screened for eligibility, and written informed consent was provided. For group 1, each subject was randomly assigned at a 1:1:1 ratio to receive two doses of 3.75 μg MF59 adjuvanted vaccines, two doses of 7.5 μg MF59 adjuvanted vaccine, or two doses of 15 μg nonadjuvanted vaccines. For group 2, each subject was randomly assigned at a 1:1 ratio to receive either two doses of 3.75 μg MF59 adjuvanted vaccine or two doses of 7.5 μg MF59 adjuvanted vaccine. Because previous study of 15 μg nonadjuvanted vaccine showed only marginal immunogenicity among the elderly (6), the 15-μg nonadjuvanted formulation was not included in group 2. Each dose was administered intramuscu-
larly into the deltoide muscle. Since the injection volumes differed between the 3.75-μg and the 7.5-μg vaccine doses, personnel who prepared and administered the study vaccine had no further involvement in the study.

The study was conducted in accordance with the Declaration of Helsinki and the standards of Good Clinical Practice proposed by the International Confer-
ence on Harmonization. The protocol and consent forms were approved by the institutional review board of each participating study site. Informed written consents were obtained from all parents and/or participants following a detailed explanation of schedules and contents of the study.

Vaccines. The influenza A (H1N1) vaccine, a monovalent, nonadjuvanted, inactivated, split-virus vaccine, was produced by Green Cross Corporation. The seed virus was prepared from reassortant virus vaccine A/California/7/2009 NYMC X-179A, which 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 sea-
sonal trivalent inactivated vaccine.

In this study, nonadjuvanted influenza vaccine was a split-virus product of 15 μg hemagglutinin antigen per 0.5-ml prefilled syringe, while MF59 adjuvanted vaccine was prepared by mixing the same amount of hemagglutinin antigen and MF59C1 (Novartis, Marburg, Germany) in a gentle, asptic manner just before injection; thus, 3.75 μg MF59 adjuvanted vaccine contained 3.75 μg hemagglu-
tinin plus 1.875 μg MF59C1 in a 0.125-ml dose, and 7.5 μg MF59 adjuvanted vaccine contained 7.5 μg hemagglutinin plus 9.75 μg MF59C1 in a 0.25-ml dose. MF59C1 consists of the following: squalene, polysorbate 80, sorbitan trioleate, trisodium citrate dehydrate, citric acid monohydrate, and water for injection (6).

Immunogenicity assessment. Sampling for immunogenicity assays was performed before the first dose (day 0), 21 days after the first vaccination (day 21), and 21 days after the second vaccination (day 42). Antibody responses were detected by hemagglutination inhibition (HI) assays according to established procedures and using turkey erythrocytes (10, 12) at the Korea University Guro Hospital. Titers of antihemagglutinin antibody 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 three coprimary immunogenicity endpoints after vaccination were as follows: the proportion of subjects with antibody titer of ≥1:40 on HI assays (seroprotection rate), the proportion of subjects with either seroconversion or a ≥4-fold increase in antibody titer (seroconversion rate), and the geometric mean titer (GMT) ratio (i.e., the ratio of the GMT after vaccination to the GMT before vaccination) (7). Serologic response, measured by HI antibody titer, was assessed using the criteria set by the Committee for Proprietary Medicinal Products (CPMP) of the European Medical Agency (EMA). To confirm protec-
tive immunogenicity, all of the following three criteria must be met: seroprotection rates of >70% for subjects aged 18 to 60 and ≥60% for subjects over 60, seroconversion rates of >40% for subjects aged 18 to 60 and ≥30% for subjects over 60, and GMT ratios of >2.5 for subjects aged 18 to 60 and >2.0 for subjects over 60.

Safety assessment. At the first visit, enrolled subjects were given a digital thermometer and a diary card containing a list of solicited adverse events and their grades. On the immunization days (day 0 and day 21), subjects were observed at the study site for a period of 30 min after vaccination to detect any immediate adverse reactions. For the next 7 days, subjects were taught how to record the severity of solicited local adverse events and systemic adverse events, axillary temperature, and concomitant medications in the diary card. Subjects used a standard scale to grade adverse events (23). The solicited local adverse events were pain, tenderness, redness, and swelling; the solicited systemic ad-
verse events were fever, headache, malaise, shivering, fatigue, sweating, myalgia,
>90% of recipients in all three vaccine regimens. In adults aged ≥65 years (group 2), on day 21 after the first dose, the rates of seroprotection and seroconversion were significantly higher following a 7.5-μg dose of MF59 adjuvanted vaccine than following a 3.75-μg dose of MF59 adjuvanted vaccine (58.0% versus 44.3% \( P = 0.03 \) and 53.7% versus 37.2% \( P < 0.01 \), respectively) (Table 2). On day 42, after two doses of vaccination, both 3.75-μg and 7.5-μg doses of MF59 adjuvanted vaccine induced very high rates of seroprotection (80.9% versus 90.9%) and seroconversion (75.5% versus 90.9%). The GMT ratio ranged between 3.5 and 27.1.

In the elderly (group 2), 7.5-μg doses of MF59 adjuvanted vaccine fulfilled the EMA criteria marginally with a single-dose vaccination, while 3.75-μg doses of MF59 adjuvanted vaccine met EMA criteria only after a two-dose priming schedule.

After the first vaccination, the GMT increased irrespective of age and vaccine regimen. However, after the second dose, GMT increment was blunted with a 15-μg dose of nonadjuvanted vaccine but increased approximately 2-fold with MF59 adjuvanted vaccines (Fig. 2). When we compared the immunogenicity levels based on preimmune status, subjects with prevaccination HI titers of ≥40 showed superior seroprotection after a single-dose immunization with either 3.75-μg doses of MF59 adjuvanted vaccine (100% versus 75.5%; \( P = 0.05 \)) or a 15-μg dose of nonadjuvanted vaccine (100% versus 76.6%; \( P = 0.14 \)) compared to those with prevaccination HI titers of <40. In comparison, the difference was not remarkable after a single 7.5-μg dose of MF59 adjuvanted vaccine (100% versus 96.6%; \( P = 0.36 \)).

Regarding preimmune status (prevaccination HI titers of ≥40 versus titers of <40), there was no significant difference in seroprotection rate after a two-dose vaccination, irrespective of vaccine formulation: 3.75-μg doses of MF59 adjuvanted vaccine (100% versus 95.1%; \( P = 0.43 \)), 7.5-μg doses of MF59 adjuvanted vaccine (100% versus 99%; \( P = 0.78 \)), and a 15-μg dose of nonadjuvanted vaccine (100% versus 89.6%; \( P = 0.21 \)).

Safety. No deaths or vaccine-related serious adverse events were reported. Five subjects were withdrawn due to non-vaccine-related adverse events, i.e., pregnancy and hospitalization (meniscus injury, herniated disc, gallbladder stone, and com-

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**TABLE 1. Demographic characteristics of the study subjects**

| Characteristic \( a \) | Value for indicated group and dose |
|--------------------------|-----------------------------------|
|                          | Group 1                      | Group 2                      |
|                          | 3.75 μg \((n = 117)\) | 7.5 μg \((n = 120)\) | 15 μg \((n = 119)\) | 3.75 μg \((n = 117)\) | 7.5 μg \((n = 117)\) |
| No. of male subjects (%) | 51 (43.6) | 54 (45.0) | 42 (35.3) | 0.26 | 54 (45.4) | 51 (43.6) | 0.78 |
| Age (yr) (mean ± SD)      | 38.7 ± 12.7 | 37.9 ± 11.7 | 40.6 ± 11.4 | 0.19 | 69.6 ± 4.2 | 70.5 ± 4.4 | 0.10 |
| BMI (mean ± SD)           | 23.0 ± 3.1 | 23.1 ± 3.2 | 23.0 ± 3.2 | 0.62 | 24.0 ± 2.7 | 24.2 ± 2.9 | 0.69 |
| No. of smokers (%)        | 24 (20.5) | 23 (19.2) | 20 (16.8) | 0.98 | 15 (12.6) | 14 (12.0) | 0.89 |
| No. of alcohol drinkers (%) | 60 (51.3) | 66 (55.0) | 59 (49.6) | 0.82 | 26 (21.9) | 24 (20.5) | 0.91 |

\( a \) Smokers were defined as subjects who smoke occasionally or daily; subjects who had been ex-smokers for <12 months were included. Alcohol drinkers were defined as subjects who drink occasionally or daily; subjects who had been ex-drinkers for <12 months were included. BMI, body mass index.

\( b \) Nonadjuvanted vaccine.
Following the first or second vaccination, though there was no statistically significant difference in overall solicited local adverse events reported within 7 days of vaccination are shown in Table 3 (group 1) and Table 4 (group 2). On the other hand, the incidences of solicited systemic adverse events were comparable between subjects with MF59 adjuvanted vaccine (191 of 473; 40.4%) and nonadjuvanted vaccine (48 of 119; 40.3%) (P = 0.99). The most common local adverse events were pain and tenderness, while the most frequent systemic event was fatigue, followed by headache and myalgia. Most subjects reported systemic adverse events as grade 1 (noticeable but did not interfere with daily activity). Overall, the frequency of solicited local adverse events was higher after injection with a 7.5-μg dose of MF59 adjuvanted vaccine (51.05%; 121/237) than after injection with a 3.75-μg dose (38.14%; 90/236) (P < 0.01). When we analyzed solicited adverse events based on age groups, local reactogenicity in the elderly (group 2) was less than half of that seen in the young adults (group 1), and the frequency of systemic adverse events was also somewhat lower in the elderly (Tables 3 and 4). As for the 15-μg dose of nonadjuvanted vaccine, no significant difference was noted in frequencies of solicited adverse events between the first and second vaccinations (data table was not shown). In comparison, MF59 adjuvanted vaccine recipients were more likely to complain of injection site pain (53.3% versus 38.9% with a 7.5-μg dose [P = 0.02]; 35% versus 28.1% with a 3.75-μg dose

### TABLE 2. Immune responses after the first and second doses of the 2009 influenza A (H1N1) vaccine, as measured by HI assay

| Immunogenicity endpoint and characteristic | Value for indicated group and dose | Adults aged 18-64 yr (group 1) | Adults aged ≥65 yr (group 2) |
|-------------------------------------------|-----------------------------------|-------------------------------|-----------------------------|
| 3.75 μg adjuvanted | 7.5 μg adjuvanted | 15 μg nonadjuvanted | P | 3.75 μg adjuvanted | 7.5 μg adjuvanted | P |
| Baseline | | | | | | |
| No. of subjects | 114 | 114 | 114 | | 113 | 112 | | | |
| % of subjects with HI titers of ≥1:40 (95% CI) | 10.5 (5.8–18.0) | 7.0 (3.3–13.8) | 6.1 (2.7–12.7) | 0.43 | 5.3 (2.2–11.7) | 5.4 (2.2–11.8) | 0.99 | | |
| GMT (95% CI) | 9.1 (7.8–10.7) | 8.3 (7.2–9.6) | 7.7 (6.9–8.6) | 0.27 | 7.8 (6.8–8.8) | 8.0 (7.1–8.9) | 0.80 | | |
| After first dose | | | | | | | |
| No. of subjects | 114 | 114 | 114 | 113 | 112 | | | | |
| % of subjects with HI titers of ≥1:40 (95% CI) | 78.1 (69.2–85.1) | 91.2 (84.1–95.5) | 78.1 (69.2–85.1) | 0.01 | 113 | 44.3 (35.0–53.9) | 58.0 (48.3–67.2) | 0.04 | |
| % of subjects with seroconversion or ≥4-fold increase in titer (95% CI) | 66.7 (57.1–75.1) | 84.2 (75.9–90.1) | 76.3 (67.3–83.6) | 0.01 | 37.2 (28.4–46.8) | 53.6 (43.9–63.0) | 0.01 | | |
| GMT (95% CI) | 77.6 (61.6–97.5) | 131.7 (107.4–161.4) | 113.1 (86.5–148.4) | 0.01 | 27.2 (21.8–33.8) | 48.5 (37.3–62.9) | <0.01 | | |
| GMT ratio (95% CI) | 8.6 (6.7–11.1) | 15.8 (12.5–19.9) | 14.7 (11.4–19.1) | <0.01 | 3.5 (2.6–4.3) | 6.1 (4.8–7.8) | <0.01 | | |
| After second dose | | | | | | | |
| No. of subjects | 113 | 112 | 113 | 110 | 110 | | | | |
| % of subjects with HI titers of ≥1:40 (95% CI) | 95.6 (89.5–98.4) | 99.1 (94.4–99.9) | 90.3 (82.9–94.8) | 0.01 | 110 | 80.9 (72.1–87.5) | 90.9 (83.5–95.3) | 0.03 | |
| % of subjects with seroconversion or ≥4-fold increase in titer (95% CI) | 89.4 (81.8–94.2) | 98.2 (93.1–99.7) | 90.3 (82.9–94.8) | 0.02 | 75.5 (66.2–82.9) | 90.9 (83.5–95.3) | <0.01 | | |
| GMT (95% CI) | 155.2 (128.0–186.0) | 226.3 (188.0–271.3) | 156.1 (123.7–196.8) | 0.01 | 76.1 (60.9–94.7) | 150.2 (119.1–188.6) | <0.01 | | |
| GMT ratio (95% CI) | 17.0 (13.5–21.3) | 27.1 (22.0–33.4) | 20.2 (16.4–25.0) | 0.01 | 9.7 (7.7–12.2) | 19.1 (15.3–23.8) | <0.01 | | |

* a Geometric mean ratios are the ratios of the antibody level obtained at the day of interest to the level obtained at day 0. Seroconversion was defined as a 4-fold increase in HI titer of 1:40 and a postvaccination titer of 1:10 and a postvaccination titer of 1:40. HI, hemagglutination inhibition; CI, confidence interval; GMT, geometric mean titer.

b The same symbols indicate nonsignificant difference between groups based on Duncan's multiple comparison test.

c The same symbols indicate nonsignificant difference between groups based on Student's t test for adults aged 18 to 64 years and Student's t test for adults aged ≥65 years.

mon bile duct stone). Solicited local adverse events and solicited systemic adverse events reported within 7 days of vaccination are shown in Table 3 (group 1) and Table 4 (group 2). Following the first or second vaccination, though there was no statistically significant difference in overall solicited local adverse events between MF59 adjuvanted vaccine (211 of 473; 44.6%) and nonadjuvanted vaccine (45 of 119; 37.8%) (P = 0.18), each solicited local adverse event was more common in subjects with MF59 adjuvanted vaccine than in those with nonadjuvanted vaccine (Table 3). On the other hand, the incidences of solicited systemic adverse events were comparable between subjects with MF59 adjuvanted vaccine (191 of 473; 40.4%) and nonadjuvanted vaccine (48 of 119; 40.3%) (P = 0.99). The most common local adverse events were pain and tenderness, while the most frequent systemic event was fatigue, followed by headache and myalgia. Most subjects reported systemic adverse events as grade 1 (noticeable but did not interfere with daily activity). Overall, the frequency of solicited local adverse events was higher after injection with a 7.5-μg dose of MF59 adjuvanted vaccine (51.05%; 121/237) than after injection with a 3.75-μg dose (38.14%; 90/236) (P < 0.01). When we analyzed solicited adverse events based on age groups, local reactogenicity in the elderly (group 2) was less than half of that seen in the young adults (group 1), and the frequency of systemic adverse events was also somewhat lower in the elderly (Tables 3 and 4). As for the 15-μg dose of nonadjuvanted vaccine, no significant difference was noted in frequencies of solicited adverse events between the first and second vaccinations (data table was not shown). In comparison, MF59 adjuvanted vaccine recipients were more likely to complain of injection site pain (53.3% versus 38.9% with a 7.5-μg dose [P = 0.02]; 35% versus 28.1% with a 3.75-μg dose

![FIG. 2. Sequential comparison of geometric mean titers (baseline, day 21, and day 42) based on age group and vaccine regimen.](image-url)
TABLE 3. Solicited local and systemic adverse events within 7 days after each vaccination among adults aged 18 to 64 years (group 1)

| Adverse event | 3.75 μg adjuvanted (n = 117) | 7.5 μg adjuvanted (n = 120) | 15 μg nonadjuvanted (n = 119) | P<sup>b</sup> |
|---------------|-------------------------------|-------------------------------|-------------------------------|---|
|                | % (95% CI) for indicated dose and grade<sup>a</sup> | % (95% CI) for indicated dose and grade | % (95% CI) for indicated dose and grade | |
| Solicited local event | | | | |
| Pain | 45.3 (36.2–54.8)<sup>b</sup> | 0 | 61.7 (52.3–70.3)<sup>b</sup> | 0 | 30.3 (22.4–39.5)<sup>b</sup> | 0.8 (0–5.3) | <0.01 |
| Tenderness | 41.0 (32.1–50.5)<sup>b</sup> | 2.6 (0.7–7.9) | 60.8 (51.5–69.5)<sup>b</sup> | 2.5 (0.7–7.7) | 31.1 (23.1–40.3)<sup>b</sup> | 1.7 (0.3–6.5) | <0.01 |
| Redness | 5.1 (2.1–11.3)<sup>b</sup> | 0.9 (0–5.4) | 11.7 (6.8–19.1)<sup>b</sup> | 1.7 (0.3–6.5) | 1.7 (0.3–6.5) | 0 | 0.01 |
| Swelling | 2.6 (0.7–7.9) | 0.9 (0–5.4) | 5.8 (2.6–12.1) | 0.8 (0–5.2) | 1.7 (0.3–6.5) | 0 | 0.24 |
| Solicited systemic event | | | | |
| Fever | 22.2 (15.3–31.0) | 1.7 (0.3–6.7) | 26.7 (19.2–35.7) | 2.5 (0.7–7.7) | 16.0 (10.1–24.1) | 1.7 (0.3–6.5) | 0.13 |
| Headache | 12.8 (7.6–20.6) | 2.6 (0.7–7.9) | 18.3 (12.1–26.7) | 3.3 (1.1–8.8) | 9.2 (4.9–16.3) | 0.8 (0–5.3) | 0.12 |
| Malaise | 12.8 (7.6–20.6) | 1.7 (0.3–6.7) | 19.2 (12.8–27.6) | 2.5 (0.7–7.7) | 9.2 (4.9–16.3) | 0.8 (0–5.3) | 0.08 |
| Fatigue | 24.8 (17.5–33.8)<sup>b</sup> | 5.1 (2.1–11.3) | 35.8 (27.4–45.2)<sup>b</sup> | 6.7 (3.1–13.1) | 21.9 (15.0–30.5)<sup>b</sup> | 0.8 (0–5.3) | 0.04 |
| Sweating | 7.7 (3.8–14.5) | 0.9 (0–5.4) | 7.5 (3.7–14.2) | 0 | 1.7 (0.3–6.5) | 0.8 (0–5.3) | 0.07 |
| Myalgia | 17.1 (11.0–25.4) | 2.6 (0.7–7.9) | 25.0 (17.8–33.9) | 4.2 (1.5–9.9) | 17.7 (11.5–25.9) | 1.7 (0.3–6.5) | 0.23 |
| Arthralgia | 6.8 (3.2–13.5) | 0.9 (0–5.4) | 6.7 (3.1–13.1) | 1.7 (0.3–6.5) | 6.7 (3.2–13.2) | 0.8 (0–5.3) | 0.99 |

<sup>a</sup> Data are presented as the proportion (% 95% confidence interval) of subjects who reported having a solicited local or systemic adverse event. Pain was graded as grade 0 (absent), grade 1 (does not interfere with activity), grade 2 (repeated use of nonnarcotic pain reliever for 24 h or interferes with activity), grade 3 (any use of narcotic pain reliever or prevents daily activity), or grade 4 (emergency room visit or hospitalization). Other adverse events were graded as follows: grade 1 (does not interfere with activity), grade 2 (interferes with activity), grade 3 (prevents daily activity), or grade 4 (emergency room visit or hospitalization).

<sup>b</sup> Comparison of all grade adverse events. Statistical significance was tested by the Chi-square test among groups.

[<P> = 0.18], myalgia (21.7% versus 10.6% with a 7.5-μg dose [<P> = 0.02]; 15.4% versus 6.1% with a 3.75-μg dose [<P> = 0.02]), and fatigue (31.7% versus 17.7% with a 7.5-μg dose [<P> = 0.01]; 18.8% versus 12.3% with a 3.75-μg dose [<P> = 0.17]) at first vaccination than at second vaccination.

Unsolicited adverse events were reported by 60 (12.7%) of 473 subjects with MF59 adjuvanted vaccine and 21 (17.7%) of 119 subjects with nonadjuvanted vaccine (P = 0.16). The most common unsolicited adverse event was respiratory symptoms (6.8 to 10.0% in young adults and 4.2 to 4.3% in the elderly), followed by musculoskeletal and gastrointestinal symptoms, but no participant reported influenza-like illness until day 42.

All subjects with unsolicited adverse events recovered without sequelae.

With regard to serious adverse events, there were 1 case of pregnancy (3.75-μg dose; group 1), 2 cases of biliary tract stone

TABLE 4. Solicited local and systemic adverse events within 7 days after each vaccination among adults aged ≥65 years (group 2)

| Adverse event | 3.75 μg adjuvanted (n = 117) | 7.5 μg adjuvanted (n = 117) | P<sup>b</sup> |
|---------------|-------------------------------|-------------------------------|---|
|                | % (95% CI) for indicated dose and grade<sup>a</sup> | % (95% CI) for indicated dose and grade | |
| Solicited local event | | | | |
| Pain | 16.0 (10.1–24.1) | 0 | 23.9 (16.7–32.9) | 0.9 (0–5.4) | 0.13 |
| Tenderness | 18.5 (12.2–26.9) | 0 | 23.1 (16.0–32.0) | 2.6 (0.7–7.9) | 0.38 |
| Redness | 3.4 (1.1–8.9) | 0.8 (0–5.3) | 6.0 (2.7–12.4) | 2.6 (0.7–7.9) | 0.34 |
| Swelling | 2.5 (0.7–7.7) | 0 | 1.7 (0.3–6.7) | 0 | 1.00 |
| Solicited systemic event | | | | |
| Fever | 20.2 (13.6–28.7) | 1.7 (0.3–6.7) | 16.2 (10.3–24.5) | 0 | 0.43 |
| Headache | 11.8 (6.8–19.3) | 0.8 (0–5.3) | 9.4 (5.0–16.6) | 0.9 (0–5.4) | 0.56 |
| Malaise | 8.4 (4.3–15.3) | 0 | 8.6 (4.4–15.5) | 1.7 (0.3–6.7) | 0.97 |
| Fatigue | 19.3 (12.9–27.8) | 1.7 (0.3–6.5) | 23.1 (16.0–32.0) | 3.4 (1.1–9.0) | 0.48 |
| Sweating | 8.4 (4.3–15.3) | 0.8 (0–5.3) | 8.6 (4.4–15.5) | 0 | 0.97 |
| Myalgia | 18.5 (12.2–26.9) | 2.5 (0.7–7.7) | 15.4 (9.6–23.5) | 3.4 (1.1–9.0) | 0.53 |
| Arthralgia | 12.6 (7.5–20.2) | 1.7 (0.3–6.5) | 15.4 (9.6–23.5) | 1.7 (0.3–6.7) | 0.54 |

<sup>a</sup> Data are presented as the proportion (% 95% confidence interval) of subjects who reported having a solicited local or systemic adverse event. Pain was graded as grade 0 (absent), grade 1 (does not interfere with activity), grade 2 (repeated use of nonnarcotic pain reliever for >24 h or interferes with activity), grade 3 (any use of narcotic pain reliever or prevents daily activity), or grade 4 (emergency room visit or hospitalization). Tenderness was graded as grade 0 (absent), grade 1 (mild discomfort to touch), grade 2 (discomfort with movement), grade 3 (significant discomfort at rest), or grade 4 (emergency room visit or hospitalization). Redness/swelling was graded as grade 0 (absent), grade 1 (2.5 to 5.0 cm), grade 2 (5.0 to 10.0 cm), grade 3 (>10.0 cm), or grade 4 (necrosis). Other adverse events were graded as follows: grade 1 (does not interfere with activity), grade 2 (interferes with activity), grade 3 (prevents daily activity), or grade 4 (emergency room visit or hospitalization).

<sup>b</sup> Comparison of all grade adverse events.
(15-μg dose; group 1), 1 case of herniated disk (3.75-μg dose; group 1), and 1 case of knee joint meniscus injury (3.75-μg dose; group 2). These events were not considered by the investigators to be vaccine related.

**DISCUSSION**

This study showed that inactivated influenza A (H1N1) monovalent vaccine was highly immunogenic in Korean adults. Pandemic influenza (2009 H1N1) vaccination was started in Korea in late October. Since then, influenza cases were markedly decreased, and overall vaccine effectiveness was estimated around 73% (21). In young adults, MF59 adjuvanted vaccine met the EMA criteria even at a single 3.75-μg dose, consistent with the report by Clark et al (5). However, in elderly subjects aged ≥65 years, a single 3.75-μg dose of MF59 adjuvanted vaccine induced a suboptimal seroprotection rate (44.3%). Considering that an HI titer of 1:40 is indicative of only a 50% reduction in the risk of influenza infection and higher titers are associated with better protection, a single ≥7.5-μg dose of MF59 adjuvanted vaccine or a two-dose priming schedule would be required for elderly subjects (17). MF59 adjuvanted influenza vaccine would be considered one of the dose-sparing strategies in the situation of vaccine shortage. Similarly, in the previous report by Roman et al., ASO3\_x (another oil-in-water adjuvant) adjuvanted influenza vaccine showed robust immunogenicity, meeting EMA criteria even at a single dose of 3.75 μg hemagglutinin antigen (20).

Levine et al. (15) compared the immune response to a single dose of nonadjuvanted influenza vaccine with the response to a two-dose vaccination (with the doses administered 8 weeks apart), which showed a similar seroprotection rate. Greenberg et al. (8) also found no difference in immune response between one-dose and two-dose immunizations with nonadjuvanted, monovalent 2009 influenza A (H1N1) vaccine. Of note, allowing for the results of this study and previous report by Cheong et al., the two-dose priming schedule showed a higher-level serologic response with the MF59 adjuvanted vaccine than with the nonadjuvanted vaccine in young adults and the elderly; Cheong et al. reported that GMTs, seroprotection rates, and seroconversion rates were indistinguishable between one-dose and two-dose immunizations with nonadjuvanted, monovalent 2009 influenza A (H1N1) vaccine (4). Considering early waning immunity in the elderly after influenza vaccination, a two-dose priming strategy with adjuvanted vaccine would be a promising option. Though the data on long-term immunogenicity with MF59 adjuvanted vaccine were not available, Nicholson et al. reported that the ASO3\_x adjuvanted vaccine induced greater seroprotective antibody titers at 6 months after vaccination than the whole-virion vaccine (16).

Allowing for the lack of preexisting antibodies cross-reactive with the novel influenza A (H1N1) virus, a two-dose vaccination was recommended for a protective antibody response in the early stage of the 2009 pandemic (3, 11, 26). Actually, in this study, even among the elderly aged ≥65 years, <10% of the study subjects had antibody titers of ≥1:40 at baseline, contrary to some reports from western countries; for example, Hancock et al. (9) reported that 34% (39 of 115 persons) born before 1950 had HI titers of ≥80. Although serologic responses to vaccination may be affected by the prevaccination titers for A/H1N1 2009, recent studies show that responses are excellent regardless of this (8, 18, 26). An immunogenicity study of an inactivated influenza A (H1N1) monovalent vaccine manufactured by CSL Limited (Parkville, Australia) demonstrated that by day 21 after the first vaccination, antibody titers of ≥1:40 were observed in 97% of study subjects who received the 15-μg dose; 31.7% of study subjects had HI titers of ≥1:40 at baseline (8). Zhu et al. (26) also reported that an HI titer of ≥1:40 was achieved by day 21 in 97.1% of subjects between 18 and 60 years and in 79.1% of subjects aged ≥61 years for a dose of 15 μg in clinical trials of an inactivated influenza A (H1N1) 2009 monovalent, split-virus vaccine (Hualan Biological Bacterin Company, Xinxian, China); about 4.0% of the study subjects had HI titers of ≥1:40 at baseline. Geographic factors in relation to possible past exposure to swine influenza outbreaks in the 1970s and/or infection with A/H1N1 2009 during the 2009-2010 influenza pandemic might affect the differences in baseline seroprevalence. This study was undertaken in the republic of Korea between October and December, before the peak of the 2009-2010 H1N1 influenza pandemic (13).

The inactivated influenza A (H1N1) 2009 monovalent vaccine was well tolerated without serious adverse events. Local adverse events were more common for the MF59 adjuvanted vaccine than for the nonadjuvanted vaccine, which were substantially greater with the 7.5-μg dose than with the 3.75-μg dose. As the levels of both the hemagglutinin antigen and MF59 were twice as high in the 7.5-μg vaccine as in the 3.75-μg vaccine, increased reactogenicity might be related to either of these. We found that local adverse events were more common with the 3.75-μg dose of MF59 adjuvanted vaccine than with the 15-μg dose of nonadjuvanted vaccine, suggesting that the MF59 adjuvant has an important role. Increased local reactogenicity was also found with other adjuvants; Roman et al. reported that the ASO3\_x adjuvanted H1N1 vaccine elicited greater local reaction than the nonadjuvanted vaccine (19). In comparison, systemic adverse events were similar and comparable to those observed with seasonal influenza vaccines (22) and other H1N1 2009 vaccines (18, 26). In line with a previous report by Beran et al., more solicited local and systemic reactions (injection site pain, fatigue, and myalgia) were reported after the first vaccination than after the second vaccination in the case of MF59 adjuvanted vaccine, which might reflect more-intense immune response after the first exposure (2).

Our study has some limitations. First, long-term follow-up was not performed; therefore, we could not observe any differences in antibody titers after 6 months between the subgroups. Second, data on previous receipt of seasonal influenza vaccines were not collected. Third, it was not possible to discern which amount was more important for immunogenicity increment, hemagglutinin contents or MF59 adjuvant. Fourth, the international antibody standard against H1N1 was not used, and there is a considerable interlaboratory variation in measuring hemagglutinin titers.

In summary, a single 7.5-μg dose of MF59 adjuvanted vaccine would have a practical advantage over a two-dose, 3.75-μg, MF59 adjuvanted vaccine priming schedule. Following a two-dose priming schedule in young adults, the increase in hemagglutinin inhibition titers was greater with MF59 adjuvanted vaccine than with nonadjuvanted vaccine. The levels of
long-term immunogenicity and clinical effectiveness of each vaccine regimen need to be compared.

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