Trial of 2009 Influenza A (H1N1) Monovalent MF59–Adjuvanted Vaccine

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ORIGINAL ARTICLE

Trial of Influenza A (H1N1) 2009 Monovalent MF59-Adjuvanted Vaccine — Preliminary Report

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ABSTRACT

BACKGROUND

The 2009 pandemic influenza A (H1N1) virus has emerged to cause the first pandemic of the 21st century. Development of effective vaccines is a public health priority.

METHODS

We conducted a single-center study, involving 175 adults, 18 to 50 years of age, to test the monovalent influenza A/California/2009 (H1N1) surface-antigen vaccine, in both MF59-adjuvanted and nonadjuvanted forms. Subjects were randomly assigned to receive two intramuscular injections of vaccine containing 7.5 μg of hemagglutinin on day 0 in each arm or one injection on day 0 and the other on day 7, 14, or 21; or two 3.75-μg doses of MF59-adjuvanted vaccine, or 7.5 or 15 μg of nonadjuvanted vaccine, administered 21 days apart. Antibody responses were measured by means of hemagglutination-inhibition assay and a microneutralization assay on days 0, 14, 21, and 42 after injection of the first dose.

RESULTS

Results of an interim analysis of the responses to the 7.5-μg dose of MF59-adjuvanted vaccine by days 14 and 21 are presented (data from four of the seven groups studied, for a total of 100 subjects). The most frequent local and systemic reactions were pain at the injection site and muscle aches, noted in 70% and 42% of subjects, respectively. Two subjects reported fever, with a temperature of 38°C or higher, after the first dosing. Antibody titers, expressed as geometric means, were generally higher at day 14 among subjects who had received two 7.5-μg doses of the MF59-adjuvanted vaccine than among those who had received only one by this time point (P=0.04 by the hemagglutination-inhibition assay and P<0.001 by the microneutralization assay). By 21 days after vaccination with the first dose of 7.5 μg of MF59-adjuvanted vaccine, the rates of seroconversion, as measured with the use of a hemagglutination-inhibition assay and a microneutralization assay, were 76% and 92% of subjects, respectively, who had received only one dose to date (with the second dose scheduled for day 21) and 88 to 92% and 92 to 96% of subjects, respectively, who had already received both doses (P=0.11 and P=0.64, respectively).

CONCLUSIONS

In preliminary analyses, the monovalent influenza A (H1N1) 2009 MF59-adjuvanted vaccine generates antibody responses likely to be associated with protection within 14 days after a single dose is administered. (ClinicalTrials.gov number, NCT00943358.)

THE EMERGENCE OF THE 2009 PANDEMIC influenza A (H1N1) virus demonstrates the unpredictable nature of influenza.[1] The 2009 H1N1 virus has the potential to cause disease, death, and socioeconomic disruption,[2,3] and mathematical modeling suggests that the effect of the virus can be reduced by immunization.[4] The development of effective vaccines is a public health priority.

Traditional seasonal influenza vaccines are produced from reassortant vaccine strains grown in hens' eggs. However, demand for vaccine against the 2009 H1N1 virus will exceed the supply if this method of manufacturing is used. Cell culture provides an alternative platform for the manufacture of influenza vaccines that may be more easily scaled up during a period of heightened demand.[5-7]

Serologic analysis suggests that after seasonal vaccination in children and adults under 50 years of age, there is little evidence of cross-reactive antibodies against the 2009 H1N1 virus,[8] with no evidence of protection from the seasonal vaccine.[9] The efficacy of conventional vaccines prepared from avian influenza strains is disappointingly low in subjects who have little evidence of cross-reactive antibodies, even if two doses are given.[10-14] The addition of oil-in-water–emulsion adjuvant enhances immunogenicity and induces cross-reactive antibodies against antigenically drifted variants.[12-16] The use of such adjuvants in influenza A (H1N1) 2009 vaccines has been suggested by the World Health Organization.[17]

Preparations for influenza A (H1N1) 2009 vaccination programs are under way, but the optimal formulation is unknown. The need for high-yield vaccine strains, limitations of the supply and production capacity of egg-based vaccines, and an expected requirement for a two-dose vaccine schedule may delay an effective immunization program.

In this preliminary report, we present the clinical-event and immunogenicity profiles of the 7.5-μg dose of the monovalent influenza A/California/2009 (H1N1) MF59-adjuvanted surface-antigen vaccine, derived from cell culture, administered to adults 18 to 50 years of age. Two doses of the vaccine were either given concurrently, one in each arm, on day 0 or one dose was given on day 0 and the other on day 7 or 14. We will update this report with findings from subjects given two doses of 3.75 μg or 7.5 μg of MF59-adjuvanted vaccine, or 7.5 μg or 15 μg of nonadjuvanted vaccine, given 21 days apart, once these data are available.

METHODS

The study was designed by one academic author and one industry author; the academic author was responsible for data management and drafting the manuscript. The data were fully accessible and interpreted by all the authors, who vouch for the accuracy and completeness of the data and analyses. The U.K. Medicines and Healthcare Products Regulatory Agency and the Leicestershire, Rutland, and Northamptonshire Ethics Committee approved the study. University Hospitals Leicester was the main sponsor; the vaccine was manufactured by Novartis, who provided funding but had no role in the conduct of the study or in preparation of the manuscript.

VACCINE

The 2009 H1N1 vaccine virus (New York Medical College [NYMC] X-179A) was generated from the influenza A/California/7/2009 strain with the use of classical reassortant methods. The gene segments encoding the hemagglutinin, neuraminidase, and the polymerase PB1 were derived from the influenza A/California/7/2009 strain, with the remaining genes taken from the influenza A/PR8/8/34 virus used as a backbone for influenza vaccines. The strain was supplied by the Centers for Disease Control and Prevention and is a pandemic vaccine strain recommended for use in vaccine development. The seed virus was grown in Madin–Darby Canine Kidney (MDCK) cell culture by means of standard processes similar to those used for the development of Optaflu vaccines against interpandemic influenza. The vaccine was formulated and produced by Novartis (Marburg, Germany) as an inactivated surface-antigen H1N1 vaccine, with or without MF59 adjuvant, and supplied in 0.5-ml prefilled single-dose syringes. Each MF59-adjuvanted vaccine contained 7.5 μg of H1 hemagglutinin, 9.75 mg of the squalene MF59, 1.175 mg of polysorbate 80, and 1.175 mg of sorbitan trioleate in buffer. Each nonadjuvanted vaccine contained 15 μg of H1 hemagglutinin in buffer. Hemagglutinin content in the final vaccine was determined by means of reverse-phase high-performance liquid chromatography, because single-radial diffusion reagents were unavailable. Vaccine was stored at 4°C until use.

STUDY DESIGN

We conducted a single-center, phase 1, randomized study from July through September 2009 at Leicester Royal Infirmary (Leicester, United Kingdom). Subjects were screened for eligibility and provided written informed consent. (For eligibility criteria, see the Supplementary Appendix, available with the full text of this article at NEJM.org.)

The first 100 subjects enrolled were randomly assigned, in a 1:1:1:1 ratio (in five blocks of 20 subjects), to receive two doses of 7.5 μg of MF59-adjuvanted vaccine, either concurrently administered on day 0 (i.e., one injection of the vaccine containing twice the antigen and adjuvant content of a single vaccine) or administered in two doses, one at day 0 and the other at day 7, 14, or 21. Serum samples for antibody measurements were collected on days 0, 14, 21, and 28. We present here the data from interim analyses performed at day 21 for these first 100 subjects.

The remaining 75 subjects enrolled were randomly assigned, in a 1:1:1 ratio (in five blocks of 15 subjects), to receive two doses of 3.75 μg of MF59-adjuvanted vaccine (by administering half the contents of the adjuvanted-vaccine syringe for each), two 15-μg doses of nonadjuvanted vaccine, or two 7.5-μg doses of nonadjuvanted vaccine (by administering half the contents of the nonadjuvanted-vaccine syringe for each) — with one injection at day 0 and the other at day 21. Serum samples were collected on days 0, 14, 21, and 42. The results for these subjects will be reported when analyses are completed.

The vaccine was administered by intramuscular injection into the deltoid muscle of the nondominant arm, or in both arms if both doses were given on day 0. Subjects were observed for 30 minutes after each injection, and for the next 7 days they recorded, in self-completed diaries, the severity of unsolicited and solicited local symptoms (pain, bruising, erythema, and swelling) and systemic symptoms (chills, malaise, muscle aches, nausea, and headache), oral temperature, and use of analgesics. Symptoms were graded as follows: none; mild, if they did not interfere with normal activities; moderate, if they resulted in interference with normal activities; and severe, if they prevented engagement in daily activities and necessitated medical attention. Serious adverse reactions were defined as any reaction that persisted beyond 7 days after vaccination or that necessitated medical attention or hospitalization during the study period.
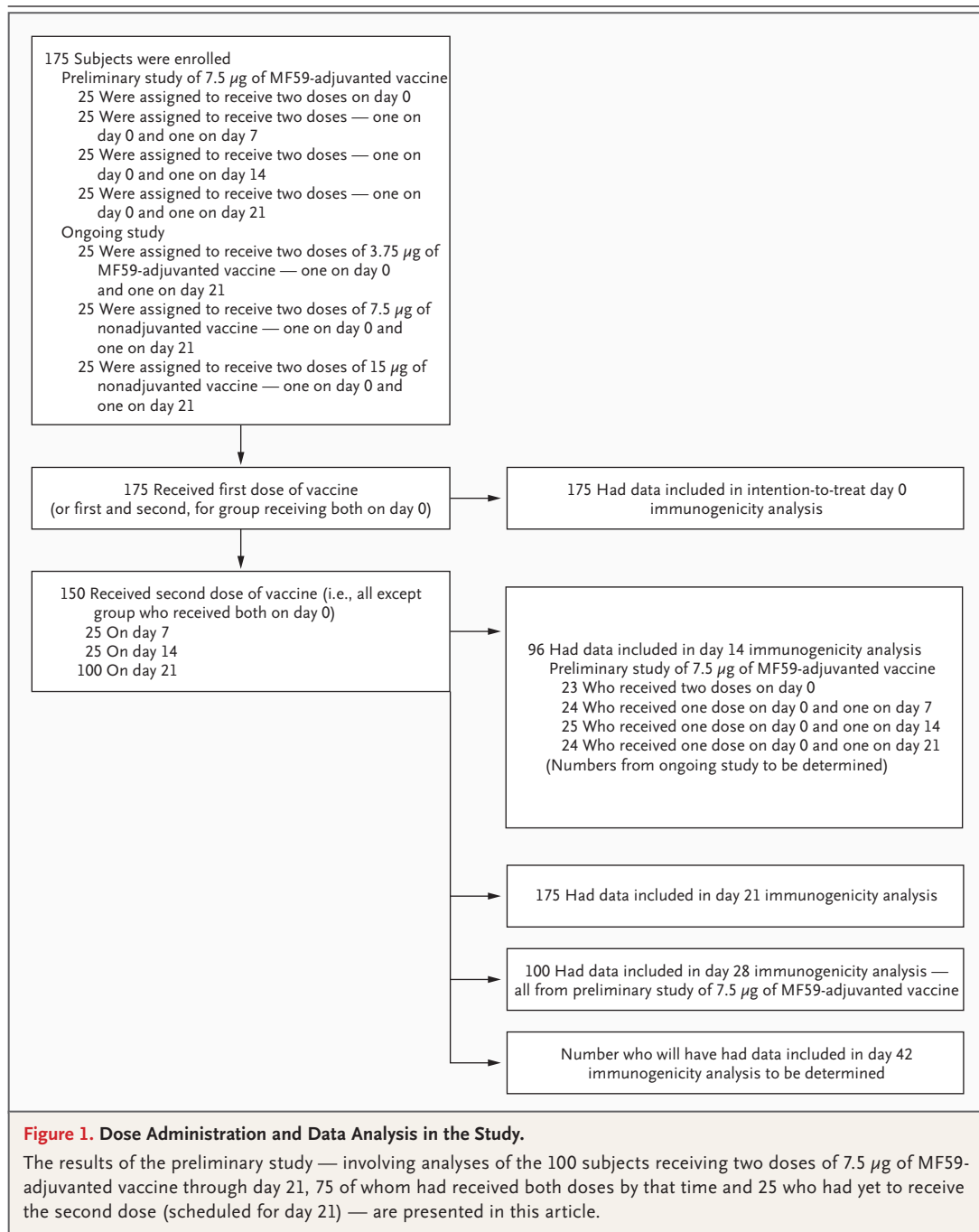
LABORATORY ASSAYS

Antibody responses were detected by means of microneutralization and hemagglutination-inhibition assays, according to standard methods,[18,19] at the Centre for Infections, Health Protection Agency (London), and with the use of cell-culture X-179A H1N1 vaccine (see the Vaccine section above), egg-grown NIBRG-121 virus, and a reverse-genetic virus containing hemagglutinin and neuraminidase from the influenza A/California/7/2009 strain (see the Supplementary Appendix for details). Serum samples obtained from subjects were tested with the use of 1:2 serial dilutions. For hemagglutination-inhibition assays, serum samples were tested at an initial dilution of 1:8, and those that were negative for the hemagglutinin antibody were assigned a titer of 1:4. Serum specimens were analyzed to determine absolute end-point titers, and the final dilution was 1:32,768. For microneutralization assays, serum samples were tested at an initial dilution of 1:10,[20] and those that were negative were assigned a titer of 1:5. The final dilution was 1:320, and samples for which the end-point titers were greater were assigned a value of 1:640. Specimens were tested in duplicate, and the geometric mean values were used in analyses.

STATISTICAL ANALYSIS

The group sizes in our study are usual for phase 1 studies and were not based on power calculations. This preliminary report details the results of analyses of data from subjects who received 7.5 μg of MF59-adjuvanted vaccine. Data analysis was undertaken with the use of Stata software (version 9.2, StataCorp).

For solicited and unsolicited adverse reactions, the percentages of subjects (point estimates and 95% confidence intervals) with postvaccination reactions were based on the frequency and severity of the reported responses after vaccination. Exact (Clopper–Pearson) confidence intervals are reported for all proportional end points. We used a two-sided Fisher's exact test to compare proportions between vaccine groups. All reported P values are two-sided, with no adjustment for multiple testing; values of 0.05 or less were considered to indicate statistical significance.

For immunogenicity analyses, the geometric mean antibody titers at each time point were used. Geometric mean titers and 95% confidence intervals were computed by taking the exponent (log$_{10}$) of the mean and of the lower and upper

175 Subjects were enrolled
    Preliminary study of 7.5 μg of MF59-adjuvanted vaccine
        25 Were assigned to receive two doses on day 0
        25 Were assigned to receive two doses — one on
            day 0 and one on day 7
        25 Were assigned to receive two doses — one on
            day 0 and one on day 14
        25 Were assigned to receive two doses — one on
            day 0 and one on day 21
    Ongoing study
        25 Were assigned to receive two doses of 3.75 μg of
            MF59-adjuvanted vaccine — one on day 0
            and one on day 21
        25 Were assigned to receive two doses of 7.5 μg of
            nonadjuvanted vaccine — one on day 0 and
            one on day 21
        25 Were assigned to receive two doses of 15 μg of
            nonadjuvanted vaccine — one on day 0 and
            one on day 21

175 Received first dose of vaccine
(or first and second, for group receiving both on day 0)

175 Had data included in intention-to-treat day 0
immunogenicity analysis

150 Received second dose of vaccine (i.e., all except
group who received both on day 0)
    25 On day 7
    25 On day 14
    100 On day 21

96 Had data included in day 14 immunogenicity analysis
    Preliminary study of 7.5 μg of MF59-adjuvanted vaccine
        23 Who received two doses on day 0
        24 Who received one dose on day 0 and one on day 7
        25 Who received one dose on day 0 and one on day 14
        24 Who received one dose on day 0 and one on day 21
    (Numbers from ongoing study to be determined)

175 Had data included in day 21 immunogenicity analysis

100 Had data included in day 28 immunogenicity analysis —
all from preliminary study of 7.5 μg of MF59-adjuvanted vaccine

Number who will have had data included in day 42
immunogenicity analysis to be determined

Figure 1. Dose Administration and Data Analysis in the Study.

The results of the preliminary study — involving analyses of the 100 subjects receiving two doses of 7.5 μg of MF59-adjuvanted vaccine through day 21, 75 of whom had received both doses by that time and 25 who had yet to receive the second dose (scheduled for day 21) — are presented in this article.

limits of the 95% confidence intervals of the $\log_{10}$-transformed titers. Geometric mean titers were compared between each pair of vaccine groups by means of one-way analysis of variance on the $\log_{10}$-transformed titers with Bonferroni correction for multiple pairwise comparisons, if appropriate. The proportions of subjects in whom seroconversion (a prevaccination hemagglutina-tion-inhibition antibody titer ≤1:10 and a post-vaccination titer ≥1:40 or a prevaccination titer ≥1:10 and an increase in the titer by a factor of four or more) or seroprotection (a hemagglutinin-inhibition antibody titer ≥1:40) was achieved were compared between each group with the use of a two-sided Fisher's exact test. Separate analy-ses were performed for the hemagglutination-

Table 1. Baseline Characteristics of the Study Subjects, According to Hemagglutinin Dose and Timing of Doses.

Characteristic	MF59-Adjuvanted Vaccine					Nonadjuvanted Vaccine	
	7.5 μg				3.75 μg	7.5 μg	15 μg
	Both Doses, Day 0 (N=25)	One Dose on Day 0, One on Day 7 (N=25)	One Dose on Day 0, One on Day 14 (N=25)	One Dose on Day 0, One on Day 21 (N=25)	One Dose on Day 0, One on Day 21 (N=25)	One Dose on Day 0, One on Day 21 (N=25)	One Dose on Day 0, One on Day 21 (N=25)
Race or ethnic group — no. (%)*							
White	21 (84)	23 (92)	19 (76)	20 (80)	17 (68)	19 (76)	25 (100)
South Asian	4 (16)	1 (4)	3 (12)	4 (16)	4 (16)	5 (20)	0
Black	0	0	3 (12)	1 (4)	3 (12)	1 (4)	0
Chinese	0	1 (4)	0	0	1 (4)	0	0
Sex — no. (%)							
Female	14 (56)	19 (76)	15 (60)	16 (64)	13 (52)	20 (80)	16 (64)
Male	11 (44)	6 (24)	10 (40)	9 (36)	12 (48)	5 (20)	9 (36)
Previous receipt of seasonal influenza vaccine — no. (%)	9 (36)	11 (44)	11 (44)	9 (36)	7 (28)	7 (28)	11 (44)
Age — yr							
Median	34	32	29	35	31	34	30
Range	19–49	18–50	23–49	20–49	20–48	24–49	24–49

* Race or ethnic group was self-reported.

inhibition and microneutralization assays. Because there are no established immune correlates for microneutralization, in that analysis we assessed the proportion of subjects who had seroconversion (an increase in the antibody titer by a factor of four or more) and a microneutralization titer of 1:40 or more.

RESULTS

This preliminary report details interim analyses through day 21 after the first vaccination of 100 subjects (four of the seven groups studied) who received two doses of 7.5 μg of MF59-adjuvanted vaccine, administered either concurrently at day 0 or as in one injection at day 0 and the other at day 7, 15, or 21 (Fig. 1). We will report the complete findings of this study when the analyses of data from all seven vaccine groups are complete, through the day 42 immunogenicity assessment.

By day 21, a total of 75 subjects had received two doses of 7.5 μg of MF59-adjuvanted vaccine (all of whom had their first dose at day 0 and 25 with the second dose on day 0, 25 with the second dose on day 7, and 25 with the second dose

on day 14). The remaining 25 subjects had received one dose, with the second dose scheduled for day 21. All subjects received their assigned vaccines, returned all diary cards, and had serum samples obtained at day 21, according to the protocol. Additional serum specimens were obtained on day 14 from 24 of the 25 subjects (96%) who had received one dose only and from 72 of the 75 (96%) who had received both doses. All 100 subjects were included in safety and immunogenicity analyses (Fig. 1).

The median age was 33 years (range, 18 to 50), 65% were female, 82% were white, and 37% had previously received seasonal influenza vaccine (Table 1). The baseline characteristics in each group were similar.

SAFETY ANALYSIS

We studied the reactogenicity after administration of the 7.5 μg of MF59-adjuvanted vaccine. Solicited local and systemic reactions during the first 7 days after the first dose of 7.5 μg of MF59-adjuvanted vaccine are shown in Table 2. Overall, 86% of subjects reported adverse reactions after either vaccine dose (85% after the first and 56%

Table 2. Solicited Local and Systemic Adverse Effects within 7 Days after Receipt of Either Dose of 7.5 μg of MF59-Adjuvanted Vaccine, According to Vaccine Group.*

Effect	One Dose on Day 0 (N = 25)†	Both Doses on Day 0 (N = 25)‡	One Dose on Day 0, One on Day 7 (N = 25)	One Dose on Day 0, One on Day 14 (N = 25)
	percent (95% confidence interval)			
Local reaction				
Pain at injection site				
None	36 (18–58)	16 (5–36)	44 (24–65)	24 (9–45)
Mild	60 (39–79)	76 (55–91)	48 (28–69)	48 (28–69)
Moderate	4 (0–20)	8 (1–26)	8 (1–26)	28 (12–49)
Severe	0 (0–14)	0 (0–14)	0 (0–14)	0 (0–14)
Redness diameter				
0 mm	96 (80–100)	88 (69–98)	88 (69–98)	84 (64–96)
1–4 mm	0 (0–14)	12 (3–31)	12 (3–31)	16 (5–36)
≥5 mm	4 (0–20)	0 (0–14)	0 (0–14)	0 (0–14)
Swelling diameter				
0 mm	96 (80–100)	84 (64–96)	96 (80–100)	80 (59–93)
1–4 mm	4 (0–20)	12 (3–31)	0 (0–14)	16 (5–36)
≥5 mm	0 (0–14)	4 (0–20)	4 (0–20)	4 (0–20)
Bruising diameter				
0 mm	96 (80–100)	92 (74–99)	92 (74–99)	84 (64–96)
1–4 mm	4 (0–20)	4 (0–20)	4 (0–20)	8 (1–26)
≥5 mm	0 (0–14)	4 (0–20)	4 (0–20)	8 (1–26)
Systemic reaction				
Muscle aches				
None	68 (47–85)	36 (18–58)	64 (43–82)	64 (43–82)
Mild§	28 (12–49)	52 (31–72)	32 (15–54)	16 (5–36)
Moderate	4 (0–20)	12 (3–31)	4 (0–20)	20 (7–41)
Severe	0 (0–14)	0 (0–14)	0 (0–14)	0 (0–14)
Chills				
None	96 (80–100)	84 (64–96)	100 (86–100)	88 (69–98)
Mild	0 (0–14)	8 (1–26)	0 (0–14)	4 (0–20)
Moderate	4 (0–20)	8 (1–26)	0 (0–14)	8 (1–26)
Severe	0 (0–14)	0 (0–14)	0 (0–14)	0 (0–14)
Malaise				
None	84 (64–96)	76 (55–91)	96 (80–100)	88 (86–100)
Mild	12 (3–31)	24 (9–46)	4 (0–20)	8 (1–26)
Moderate	4 (0–20)	0 (0–14)	0 (0–14)	4 (0–20)
Severe	0 (0–14)	0 (0–14)	0 (0–14)	0 (0–14)
Headache				
None	68 (47–85)	60 (39–79)	80 (59–93)	56 (35–76)
Mild	28 (12–49)	36 (18–58)	12 (3–31)	28 (12–49)
Moderate	4 (0–20)	4 (0–20)	8 (1–26)	16 (5–36)
Severe	0 (0–14)	0 (0–14)	0 (0–14)	0 (0–14)

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Effect	One Dose on Day 0 (N=25)†	Both Doses on Day 0 (N=25)‡	One Dose on Day 0, One on Day 7 (N=25)	One Dose on Day 0, One on Day 14 (N=25)
Table 2. (Continued.)				
		percent (95% confidence interval)		
Nausea				
None	80 (59–93)	88 (69–98)	92 (74–99)	84 (64–96)
Mild	12 (3–31)	12 (3–31)	4 (0–20)	12 (3–31)
Moderate	8 (1–26)	0 (0–14)	4 (0–20)	4 (0–20)
Severe	0 (0–14)	0 (0–14)	0 (0–14)	0 (0–14)
Fever, temperature ≥38°C	0 (0–14)	4 (0–20)	0 (0–14)	4 (0–20)
Use of analgesic	8 (1–26)	20 (7–41)	0 (0–14)	16 (5–36)

* Subjects used a subjective scale to grade adverse events. Symptoms were considered mild if they did not interfere with daily activities, moderate if they caused some impairment, and severe if they affected daily activities and necessitated medical attention.
† The group who had received one dose on day 0 had the second dose scheduled for day 21, but it had not yet been administered.
‡ For the group who had received both doses concurrently on day 0, any local reaction in either arm is reported.
§ Muscle aches were reported significantly more frequently in the group receiving both doses on day 0 than in the other three groups (P=0.02 by Fisher's exact test).

after the second). The frequency or severity of reactions did not increase after the second vaccine as compared with after the first (see the Supplementary Appendix). All self-reported reactions were graded as mild or moderate and were generally self-limited, resolving within a 72-hour period.

The most frequent local reaction after any dose of 7.5 μg of MF59-adjuvanted vaccine was pain at the injection site, in 70% of subjects. In general, such pain was not accompanied by redness or swelling, although one subject recorded bruising, in an area 20 mm in diameter, after the first dose, with resolution within 72 hours. No severe local reactions were reported.

The most frequent systemic reaction was muscle ache, in 42% of subjects. A total of 11% of subjects reported using an analgesic for systemic symptoms. Two subjects reported fever, defined as temperature of 38°C or more, after receipt of the first dose, but neither used antipyretic medication. In general, the frequency and severity of solicited reactions after injection of the 7.5 μg of MF59-adjuvanted vaccine did not differ among the groups, although subjects who received both doses on day 0 reported a greater frequency of systemic reactions (muscle aches) as compared with the other three groups (P=0.02). No severe systemic reactions were reported.

Seven unsolicited adverse events were reported. Three subjects reported self-limiting diarrhea that resolved within the 48-hour period after the first dose; one subject took over-the-counter loperamide as treatment. Two subjects reported coryza that resolved within 48 hours. One subject reported a toothache that resolved after 5 days. One subject reported a transient itchy rash on the right forearm that resolved within 48 hours (Table 3 in the Supplementary Appendix).

A probable vaccine-related adverse reaction, reported after receipt of the 7.5 μg of MF59-adjuvanted vaccine, is described in the Supplementary Appendix. Briefly, this subject (who received two doses of the MF59-adjuvanted vaccine on day 0) reported a purpuric rash on the lower limbs on day 17, with resolution within 72 hours. Further questioning revealed that she had consulted with her family practitioner in May 2009 for an intermittent leg rash within the 12-month period before the study. Investigations including complete blood count and biochemical profile showed normal values, but an autoimmune profile was positive for antinuclear and extractable nuclear-antigen antibodies. She had not received medication, and this previous medical history was not known at enrollment. Follow-up for the rash included a normal complete blood count and biochemical profile. Results of autoimmune testing were unchanged from those in May 2009.

IMMUNOGENICITY AGAINST THE 2009 H1N1 VIRUS

Interim analysis was conducted at day 21 after the first dose of 7.5 μg of MF59-adjuvanted vaccine.

Table 3. Antibody Responses to 7.5 µg of MF59-Adjuvanted Vaccine, as Measured with the Hemagglutination-Inhibition Assay, According to Vaccine Group.*

Value	One Dose on Day 0 (N=25)†	Both Doses on Day 0 (N=25)	One Dose on Day 0, One on Day 7 (N=25)	One Dose on Day 0, One on Day 14 (N=25)
Day 0				
Geometric mean titer (95% CI)	6.2 (4.0–9.4)	6.0 (3.8–9.5)	4.8 (3.7–6.3)	6.6 (4.2–10.4)
Seroprotection — % (95% CI)	12 (3–31)	8 (1–26)	4 (0–20)	12 (3–31)
Day 14‡				
Geometric mean titer (95% CI)	195.6 (88.8–431.0)	294.8 (165.9–523.5)	416.5 (260.9–664.9)	155.8 (62.7–387.2)
Geometric mean ratio (95% CI)	31.7 (13.7–73.5)	49.2 (24.6–98.2)	86.7 (52.3–143.8)	23.7 (8.9–62.2)
Seroconversion — % (95% CI)	79 (58–93)	91 (72–99)	96 (79–100)	68 (47–85)
Seroprotection — % (95% CI)	83 (63–95)	96 (78–100)	100 (86–100)	72 (51–88)
Day 21				
Geometric mean titer (95% CI)	172.5 (80.2–370.8)	256.1 (158.0–415.2)	282.9 (160.2–499.7)	288.7 (150.6–553.7)
Geometric mean ratio (95% CI)	27.9 (12.2–64.2)	42.7 (22.7–80.5)	58.9 (32.4–107.0)	43.8 (20.6–93.2)
Seroconversion — % (95% CI)	76 (55–91)	88 (69–98)	92 (74–99)	88 (69–98)
Seroprotection — % (95% CI)	80 (59–93)	92 (74–99)	96 (80–100)	92 (74–99)

* Geometric mean titers are the ratios of the antibody level at the day of interest and at day 0. Percentages of subjects are based on the total number of subjects tested. Seroprotection was defined as an antibody titer of 1:40 or more. Seroconversion was defined as prevaccination antibody titer of 1:10 or less and a postvaccination titer of 1:40 or more or a prevaccination titer greater than 1:10 and an increase in the antibody titer by a factor of four or more.

† The group that had received one dose on day 0 had the second dose scheduled for day 21, but it had not yet been administered.

‡ At day 14, the geometric mean ratio, the rate of seroconversion, and the rate of seroprotection were all significantly greater (P=0.04, P=0.01, and P=0.04, respectively) in the two groups that had received two doses (the first dose on day 0 and the second dose on day 0 or day 7) as compared with the two groups that had received one dose only (with the second dose scheduled for day 14 or day 21).

Antibody responses against the vaccine were measured for each of the four groups.

Antibodies were detected, as measured by hemagglutination-inhibition assay (titer >1:8) and microneutralization assay (titer >1:10) before vaccination in 14% and 39% of subjects, respectively, with this frequency unrelated to age (P=0.91 by hemagglutination-inhibition assay and P=0.48 by microneutralization assay) or previous receipt of seasonal vaccine (P=0.15 and P=0.39, respectively).

On day 14, geometric mean titers, as measured with the use of hemagglutination-inhibition assay (Table 3) and microneutralization assay (Table 4), were higher in subjects who received two doses of 7.5 µg of MF59-adjuvanted vaccine by this time, as compared with those who had received one dose only (P=0.04 by hemagglutination-inhibition assay and P<0.001 by microneutralization assay), but there was no significant difference in titer among the groups (P=0.34 by hemagglutination-inhibition assay and P=0.10 by microneutralization assay). On day 21, there was no significant difference in titer among subjects who had received one dose of 7.5 µg of MF59-adjuvanted vaccine and those who had received two (P=0.18 by hemagglutination-inhibition assay and P=0.051 by microneutralization assay).

Table 3 shows the ratio of antibody titer after first vaccination and before first vaccination and the percentages of subjects with seroconversion and seroprotection, as measured by means of the hemagglutination-inhibition assay. On day 14, the rates of seroconversion and seroprotection were higher (P=0.01 and P=0.004, respectively) among subjects who had two doses than among those who had only one. On day 21, there was no significant difference in the rate of seroconversion (P=0.11) or seroprotection (P=0.12) among those who had received one and those who had received two doses.

There was also no significant difference in the rate of seroconversion between subjects who had antibody detected at baseline and those who did

Table 4. Antibody Responses to 7.5 μg of MF59-Adjuvanted Vaccine, as Measured with the Microneutralization Assay, According to Vaccine Group.*

Value	One Dose on Day 0 (N = 25)†	Both Doses on Day 0 (N = 25)	One Dose on Day 0, One on Day 7 (N = 25)	One Dose on Day 0, One on Day 14 (N = 25)
Day 0				
Geometric mean titer (95% CI)	13.4 (8.2–21.9)	10.4 (5.9–18.1)	9.8 (6.6–14.5)	13.1 (7.8–22.1)
Antibody titer ≥40 — % (95% CI)	20 (7–41)	12 (3–31)	16 (5–36)	16 (5–36)
Day 14				
Geometric mean titer (95% CI)‡	353.3 (256.5–486.5)	606.5 (557.8–659.5)	502.2 (406.7–622.6)	285.4 (185.1–439.9)
Seroconversion — % (95% CI)	83 (63–95)	91 (72–99)	96 (79–100)	84 (64–96)
Antibody titer ≥40 — % (95% CI)	100 (86–100)	100 (85–100)	100 (86–100)	92 (74–99)
Day 21				
Geometric mean titer (95% CI)	348.2 (247.3–490.3)	582.8 (518.3–655.3)	448.9 (335.1–601.5)	407.2 (301.4–550.3)
Seroconversion — % (95% CI)	92 (74–99)	92 (74–99)	96 (80–100)	96 (80–100)
Antibody titer ≥40 — % (95% CI)	100 (86–100)	100 (86–100)	100 (86–100)	100 (86–100)

* Percentages of subjects are based on the total number of subjects tested. Because no threshold titer for seroprotection has been established for the microneutralization assay, the percentage of subjects with titers of 1:40 or greater are reported. "Antibody titer ≥40" denotes a titer of 1:40 or greater at each postvaccination visit. Seroconversion was defined as an increase in the antibody titer by a factor of four or more.

† The group who had received one dose on day 0 had the second dose scheduled for day 21, but it had not yet been administered.

‡ At day 14, the geometric mean ratio was significantly greater (P=0.04) in the two groups who had received two doses (the first dose on day 0 and the second dose on day 0 or day 7) as compared with the two groups that had received one dose only (with the second dose scheduled for day 14 or day 21).

not (P=0.68 by the hemagglutination-inhibition assay and P=0.66 by the microneutralization assay). Because various dosing intervals were evaluated, postvaccination responses at specific time points may not reflect overall antibody kinetics.
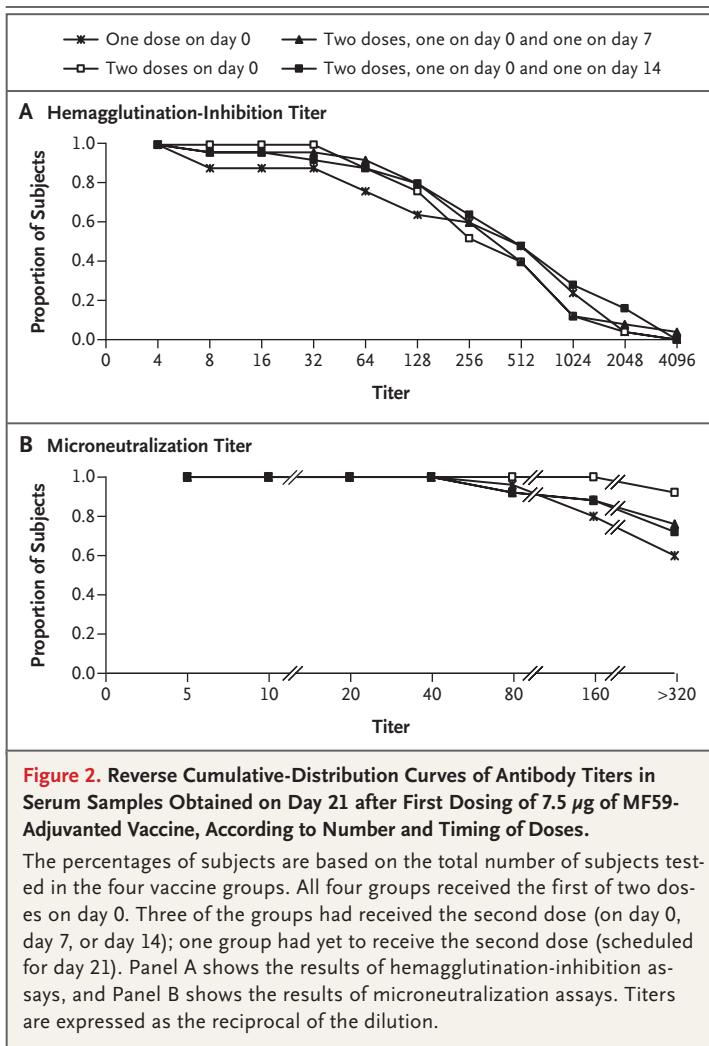
Table 4 shows the percentages of subjects with seroconversion and antibody titers exceeding 1:40, as measured by means of the microneutralization assay. On days 14 and 21, there was no significant difference among the vaccine groups in the rate of seroconversion (day 14, P=0.20; day 21, P=0.64) or those with titers exceeding 1:40 (day 14, P=0.50; day 21, P=1.00). Figure 2 shows the distribution of antibody titers at day 21 after the first dose, according to vaccine group. Titers of the hemagglutination-inhibition antibody exceeded 1:32 in 88% of subjects who had received one vaccine dose by this time and in 92 to 100% of subjects who had received both doses (Fig. 2A). All subjects had microneutralization antibody at a titer exceeding 1:40 by day 21 (Fig. 2B).

Responses, as measured with the use of hemagglutination-inhibition assay, against the NIBRG-121 virus were similar to those against the 2009 H1N1 virus (see the Supplementary Appendix).

DISCUSSION

This report details the interim findings for subjects receiving the 7.5-μg dose of the MF59-adjuvanted vaccine. We found that the immune responses were consistent with seroprotection against the 2009 pandemic influenza A (H1N1) virus within 2 weeks after administration of a single dose of the adjuvanted cell-culture vaccine. Data for the recipients of nonadjuvanted vaccine will be reported when analysis is completed.

Effective vaccination should reduce morbidity, mortality, and virus transmission,[4] although this is challenging to achieve. The global demand for vaccine will likely exceed manufacturing capacity and will only be met by implementing a range of approaches to vaccine development. Large-scale vaccine production with the use of newly characterized viruses can be challenging if low egg growth limits the supply of antigen. Our vaccine was produced from a classical egg-derived seed virus propagated in a MDCK cell line.[7,21] Cell-culture systems may provide a faster response and potentially greater scale-up than egg production. Cell-culture seasonal influenza seed viruses also

Figure 2. Reverse Cumulative-Distribution Curves of Antibody Titers in Serum Samples Obtained on Day 21 after First Dosing of 7.5 μg of MF59-Adjuvanted Vaccine, According to Number and Timing of Doses.

The percentages of subjects are based on the total number of subjects tested in the four vaccine groups. All four groups received the first of two doses on day 0. Three of the groups had received the second dose (on day 0, day 7, or day 14); one group had yet to receive the second dose (scheduled for day 21). Panel A shows the results of hemagglutination-inhibition assays, and Panel B shows the results of microneutralization assays. Titers are expressed as the reciprocal of the dilution.

show better antigenic matching to clinical isolates than egg-passaged vaccine strains.[5-8,21]

Clinical experience with avian and human influenza A/H1N1–subunit vaccines in immunologically naive subjects who had little evidence of cross-reactive antibodies suggests that two doses are required to induce seroprotection.[10-15,22,23] Traditionally, dosing intervals of 21 to 28 days are used, often delaying effective immunization. In our interim analysis, we report data on several immunization schedules involving two 7.5-μg doses of MF59-adjuvanted vaccine. Flexible dosing would be useful for authorities organizing immunization regimens, although a single vaccination against the 2009 pandemic influenza A (H1N1) virus would be logistically most desirable.

Our findings add to observations that oil-in-water–emulsion adjuvants have short-term reacto-

genicity equivalent to that of inactivated seasonal vaccines.[24] Although adjuvants generally enhance immunogenicity, their effect on the 2009 H1N1 virus is unknown. Our head-to-head evaluation with nonadjuvanted formulations will assess the effect of adding MF59 adjuvant on the speed and magnitude of antibody responses. For avian-influenza subvirion vaccines, oil-in-water–emulsion adjuvants are essential to inducing long-lasting cross-reactive immunity.[10,12-15] Although influenza A (H1N1) virus isolates are antigenically homogeneous, the induction of antibody that is cross-reactive to drift variants would be a desirable characteristic of the first vaccines against the 2009 H1N1 virus.

Interpretation of immunogenicity data for the vaccine against the 2009 pandemic influenza A (H1N1) virus is complicated by a lack of recognized immune correlates. The insensitivity of hemagglutination-inhibition assays to some avian hemagglutinin has required that microneutralization assays, hemagglutination-inhibition assays involving horse erythrocytes, or single radial hemolysis be used.[10,18,25] Because there is significant laboratory variation in testing,[20] efforts to develop biologic standards for serologic assays of influenza A (H1N1) viruses are under way. Fifteen percent of subjects had detectable prevaccination levels of hemagglutination-inhibition antibody, consistent with results of seroepidemiologic studies.[11] Although we excluded subjects with previous respiratory illnesses, asymptomatic infection with influenza A (H1N1) viruses cannot be ruled out, as local activity was present during the study.

The safety and immunogenicity of these and alternative candidate vaccines against the 2009 H1N1 virus, including egg-derived, whole-virion, recombinant, and live-attenuated vaccines must be assessed in high-risk populations, including children, the elderly, and other persons whose immunologic profiles may differ from those of young adults.[8] In addition, the duration of antibody responses and their ability to be boosted after revaccination should be established to predict protection against future pandemic waves.

Finally, although seasonal influenza vaccines have an established safety profile, there are occasional case reports of unusual reactions, including vasculitis.[24,26] MF59, a proprietary oil-in-water–emulsion adjuvant, was first licensed for use in seasonal influenza vaccines in 1997. Over 40 million doses have been delivered in Europe, and over 16,000 doses administered in clinical trials, with

no excess reports of autoimmune conditions.[27] It is important to ensure post-marketing surveillance during any mass use of a pandemic-virus vaccine, with or without adjuvant.

This preliminary report of vaccines against the 2009 pandemic influenza A (H1N1) virus with or without MF59 will be updated as analyses are completed but may be useful for the planning of immunization schedules and future trials and comparisons with other vaccine options as they become available.

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