INFLUENZA

A Cell Culture–Derived MF59-Adjuvanted Pandemic A/H7N9 Vaccine Is Immunogenic in Adults

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A potentially deadly A/H7N9 avian-origin influenza virus is currently the cause of an ongoing outbreak in China. Preparedness plans have thus been initiated to preempt the spread of this virus, which appears to have substantial pandemic potential. To effectively prevent a pandemic from unfolding, rapid production of an immunogenic vaccine with an acceptable safety profile is critical. Given the significance to public health, we are reporting immunogenicity and safety results from a phase 1 study in healthy adults administered one of four inactivated A/H7N9 vaccine formulations. Three formulations contained increasing quantities of antigen and of an oil-in-water adjuvant, MF59, and one formulation contained only the maximum dose of antigen without adjuvant. All vaccine formulations were derived using a synthetic virus seed technology in combination with a cell culture approach; together, these techniques have been shown to expedite vaccine production compared to conventional methods. Higher responses were seen with the MF59-adjuvanted versus the nonadjuvanted A/H7N9 vaccine, with significant and potentially protective immune responses after two doses in most subjects with no preexisting immunity to the H7N9 virus. Further, despite increased injection site pain and other mild effects with MF59, all formulations were well tolerated. These encouraging immunogenicity and safety data on the A/H7N9 vaccine provide a strong rationale for further clinical development. By also using synthetic seed/cell culture technology, we are now one step closer to being able to rapidly and reliably respond to a potential H7N9 pandemic using a clinically tested A/H7N9 vaccine.

INTRODUCTION

A potentially deadly A/H7N9 strain of avian influenza emerged in China in February 2013 (*1*), causing 135 reported cases of human disease by August 2013. It has since reemerged for a second, more severe season with the onset of cooler weather, leading to a total of 375 confirmed cases and 155 deaths to date (*2*). This A/H7N9 influenza virus is antigenically distinct from the circulating seasonal influenza viruses. Therefore, standard seasonal vaccines are not considered protective against A/H7N9 infection (*3*). Although occasional reports on the transmission of other avian H7 viruses to mammals exist in the literature, no cases of A/H7N9 influenza had been documented in humans before 2013 (*4*). Consequently, most of the population is believed to be susceptible to the avian A/H7N9 virus, and the potential spread of this virus poses a significant concern to public health. To date, although this A/H7N9 virus has not shown sustained transmissibility between humans (*5*), an epidemiologic study from March 2013 did report limited human-to-human spread between two family members in China (*6*). In addition, recent experimental evidence found that the A/H7N9 virus is transmissible between ferrets via respiratory droplet (*7*). Going forward, should this H7N9 virus mutate to become more transmissible between humans, it is anticipated that large-scale national vaccination programs will be initiated to preempt an A/H7N9 influenza pandemic.

Extensive efforts are therefore under way to develop effective immunogenic vaccines targeted against the A/H7N9 virus rapidly. Unfortunately, conventional egg-derived vaccines can suffer from a variety of manufacturing problems, including microbial contamination and poor growth of some human influenza viruses in eggs, leading to potential delays in vaccine supply. Cell culture–derived vaccines manufactured from a seed virus (*8*, *9*), however, could be produced rapidly, allowing swift responses to changes in demand. In addition, because fully cell culture–derived influenza vaccines are more likely to preserve the antigenic structure of the hemagglutinin (HA) antigens, they may even be superior to egg-based vaccines (*10*). To date, a Madin-Darby canine kidney (MDCK) cell culture technology has already been licensed for the manufacture of seasonal influenza vaccines in the United States (Flucelvax, Novartis Vaccines), as well as for both seasonal (Optaflu, Novartis Vaccines) and pandemic vaccines (Celtura, Novartis Vaccines) in Europe. Moreover, in a recent simulated (timed) response to a potential influenza pandemic, the use of a synthetic seed virus, containing the HA and neuraminidase (NA) genes from a supplied A/H7N9 virus sequence, was investigated in conjunction with the MDCK cell culture technology. Together, these approaches resulted in impressively rapid vaccine production rates, much faster than currently possible with standard methods (*8*). These technologies were thus adopted to generate a cell culture–derived H7N9 vaccine (H7N9c) for this phase 1 trial. The formulations tested were based on A/Shanghai/2/2013 HA and NA gene sequences that had been posted (early in the A/H7N9 influenza outbreak) on the Global Initiative for Sharing All Influenza Data platform by the China Centers for Disease Control.

Although the prompt manufacture of an appropriate vaccine is clearly desirable during a pandemic, to ensure adequate vaccine supplies, antigen-sparing preparations exhibiting maximum immunogenicity are equally critical. Unfortunately, clinical trials to date examining the immunogenicity of various H7-specific vaccines have shown relatively poor results (*5*). Modest immune responses have also been seen with other avian-origin prepandemic influenza vaccines and have been successfully overcome by the use of adjuvants (*11*). Adjuvants have the double benefit of both enhancing immune responses to influenza vaccines and economizing on the use of antigen. Indeed, the World

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Health Organization actively endorsed the inclusion of adjuvants in the 2009 A/H1N1 pandemic vaccines (*12*). MF59 (Novartis Vaccines) is a well-established oil-in-water emulsion adjuvant shown to significantly augment the immune response to both pandemic and seasonal influenza vaccines licensed in the European Union and other countries (*13*, *14*). In addition, MF59 has a positive safety profile with more than 100 million doses of MF59-adjuvanted influenza vaccines distributed to date (*15*–*17*). MF59 is thus an attractive adjuvant for a potential A/H7N9 vaccine.

Because of the public health importance of the current A/H7N9 outbreak and the need for pandemic preparedness, we are reporting promising results on the immunogenicity and safety of four candidate inactivated A/H7N9 monovalent subunit influenza pandemic vaccines. Formulations containing increasing amounts of synthetic virus–based, mammalian cell culture–derived H7N9 antigen and varying amounts of MF59 were examined in adults aged between 18 and 64 years.

RESULTS

A total of 402 subjects were enrolled in a phase 1, U.S. multicenter, observer-blind, randomized study to assess the safety and immunogenicity of investigational formulations of a MF59-adjuvanted, cell-based, inactivated A/H7N9 monovalent subunit influenza virus vaccine (H7N9c) in healthy adult subjects in the United States. Enrolled subjects were randomized equally into the following four groups, each to receive two doses, administered 3 weeks apart: group A, 3.75 μg of A/H7N9 HA + 0.125 ml of MF59; group B, 7.5 μg of HA + 0.25 ml of MF59; group C, 15 μg of HA + 0.25 ml of MF59; and group D, 15 μg of HA without MF59. Antibody responses to the H7N9c vaccines were assessed 3 weeks after each dose (days 22 and 43) by hemagglutination inhibition (HI) and microneutralization (MN) assays. This analysis was conducted on all data to day 43, 3 weeks after administration of the second dose, to evaluate the immunogenicity, reactogenicity, and safety of both the MF59-adjuvanted and non-adjuvanted H7N9c vaccine formulations. Immunogenicity data from 396 of 402 subjects were eligible for analysis on day 43. Six subjects were excluded from this analysis either because they received no vaccination (n = 1) or because they did not receive the second dose of the vaccination (n = 5). Safety data from all subjects receiving any vaccination (n = 401) were assessed on day 43. Figure 1 illustrates the design of the study as a flow chart.

Table 1 displays the demographics and baseline characteristics of the enrolled subjects (n = 402). The mean age of subjects for each group ranged from 38.3 to 43.4 years; this difference was not statistically significant. The male-to-female ratios across treatment groups likewise showed only small variations, with group A having a slightly higher proportion of female subjects. About 81% of the study population was Caucasian.

MF59-adjuvanted H7N9c vaccine has a dose-dependent effect on immune response by day 43

On day 1 of the study, all subjects that provided evaluable sera (n = 401) had undetectable (<1:10) HI titers against A/H7N9 influenza. As expected for an immunologically naïve population, responses to the

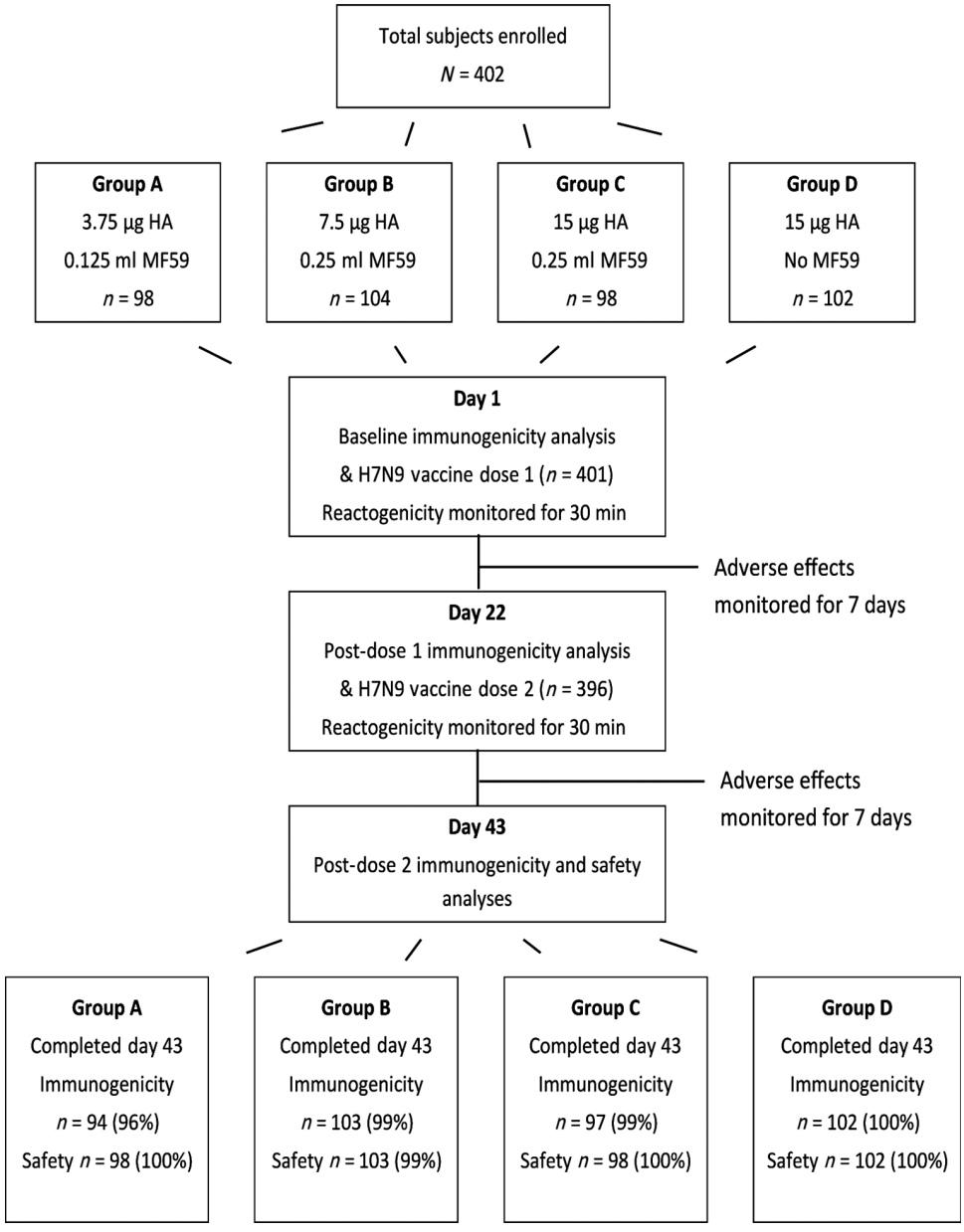

Fig. 1. Flow chart of study design.

first dose were minimal on day 22. By day 43, however, a dose-response effect was seen in the MF59-adjuvanted groups (Table 2 and Figs. 2 and 3). Specifically, for groups A to C, the HI geometric mean titers (GMTs) on day 43 increased with antigen and adjuvant content, with values rising from 12 (group A) to 26 (group C); in contrast, the GMT for the nonadjuvanted group D remained low at 5.8. GMT levels in the MN assay mirrored those of the HI assay, but the dose-dependent trend was even more pronounced. Indeed, MN GMT levels across the groups already showed a trend toward dose dependency by day 22. By day 43, this trend increased considerably, with GMT values for groups A to C ranging from 42 to 100, whereas group D achieved only a modest increase in GMT to 7.7.

HI titers of ≥1:40 are widely recognized as an immunologic correlate in adults corresponding to a 50% reduction in the risk of contracting seasonal influenza (*18*, *19*). Although a "seroprotective" correlate has not been established for pandemic influenza strains (including A/H7N9), HI titers of ≥1:40, or MN titers of ≥1:20 (*20*, *21*) or ≥1:40 (*22*, *23*), are often used as reference points for assessing the immunogenic potential of prepandemic influenza vaccines. We thus investigated the percentage of subjects with a titer of ≥1:40 using the HI (Fig. 2A) and MN (Fig. 2B) assays and found that both showed a clear dose-dependent trend on day 43. Specifically, the percentage of subjects in the dose-escalating, MF59-adjuvanted groups A, B, and C reaching an HI titer of ≥1:40 was 26, 44, and 52%, respectively. In contrast, the percentage of subjects in the nonadjuvanted vaccine group D was only 3%. MN analyses showed similar results with 55, 73, and 78% of subjects exhibiting a titer of ≥1:40 on day 43 for groups A, B, and C, respectively, whereas only 7% of subjects in group D exhibited this level of response.

In parallel to measuring the percentage of subjects with titers of ≥1:40 (Fig. 2), we also assessed the percentage of subjects exhibiting a fourfold or greater increase in HI (Fig. 3A) and MN (Fig. 3B) titers from baseline, which is a commonly used criterion of "seroconversion." As expected of a population with no previous exposure, and thus lacking baseline seropositivity, to the A/H7N9 virus, the percentages of subjects exhibiting a minimum fourfold increase in HI and MN titers (Fig. 3) were almost identical to the respective group percentages seen in Fig. 2. Collectively, these results indicate that a significant and possibly protective antibody response can be elicited after two doses of MF59-adjuvanted H7N9c vaccine formulations but not with the nonadjuvanted H7N9c vaccine formulation.

MF59-adjuvanted H7N9c vaccine is well tolerated

Over the course of the study, about 68 and 54% of all subjects experienced a solicited adverse effect (AE) after the first and second vaccinations, respectively. As expected, there were a higher number of

Table 1. Demographics of enrolled subjects.

	Group A (*n* = 98)	Group B (*n* = 104)	Group C (*n* = 98)	Group D (*n* = 102)	Total (*n* = 402)
Vaccine components					
HA content (μg)	3.75	7.5	15	15	
MF59 content (ml)	0.125	0.25	0.25	0	
Age (years ± SD)	38.3 ± 13.1	40.3 ± 12.5	42.6 ± 13.1	43.4 ± 12.9	41.2 ± 13.0
Gender [*n* (%)]					
Female	60 (61)	57 (55)	48 (49)	53 (52)	218 (54)
Male	38 (39)	47 (45)	50 (51)	49 (48)	184 (46)
Race [*n* (%)]					
White	77 (79)	88 (85)	77 (79)	85 (83)	327 (81)
Non-white	21 (21)	16 (15)	21 (21)	17 (17)	75 (19)

Table 2. GMTs and 95% CIs for the HI and MN assays on days 1, 22, and 43.

	Group A		Group B		Group C		Group D	
	n	3.75 μg of HA + 0.125 ml of MF59	*n*	7.5 μg of HA + 0.25 ml of MF59	*n*	15 μg of HA + 0.25 ml of MF59	*n*	15 μg of HA no MF59
HI assay titer (95% CI)								
GMT day 1	98	5.00 (4.95–5.05)	103	5.00 (4.95–5.05)	98	5.00 (4.95–5.05)	102	5.05 (5.00–5.1)
GMT day 22	96	5.07 (4.95–5.2)	103	5.00 (4.88–5.12)	97	5.00 (4.88–5.13)	102	5.1 (4.98–5.23)
GMT day 43	94	12 (9.75–15)	103	19 (15–23)	97	26 (21–32)	102	5.75 (4.67–7.08)
MN assay titer (95% CI)								
GMT day 1	98	5.08 (4.90–5.27)	103	5.26 (5.08–5.45)	98	5.08 (4.9–5.27)	102	5.08 (4.9–5.26)
GMT day 22	96	5.69 (5.14–6.31)	103	6.16 (5.58–6.79)	97	7.07 (6.39–7.83)	102	5.3 (4.81–5.86)
GMT day 43	94	42 (33–54)	103	76 (60–95)	97	100 (79–128)	102	7.7 (6.09–9.73)

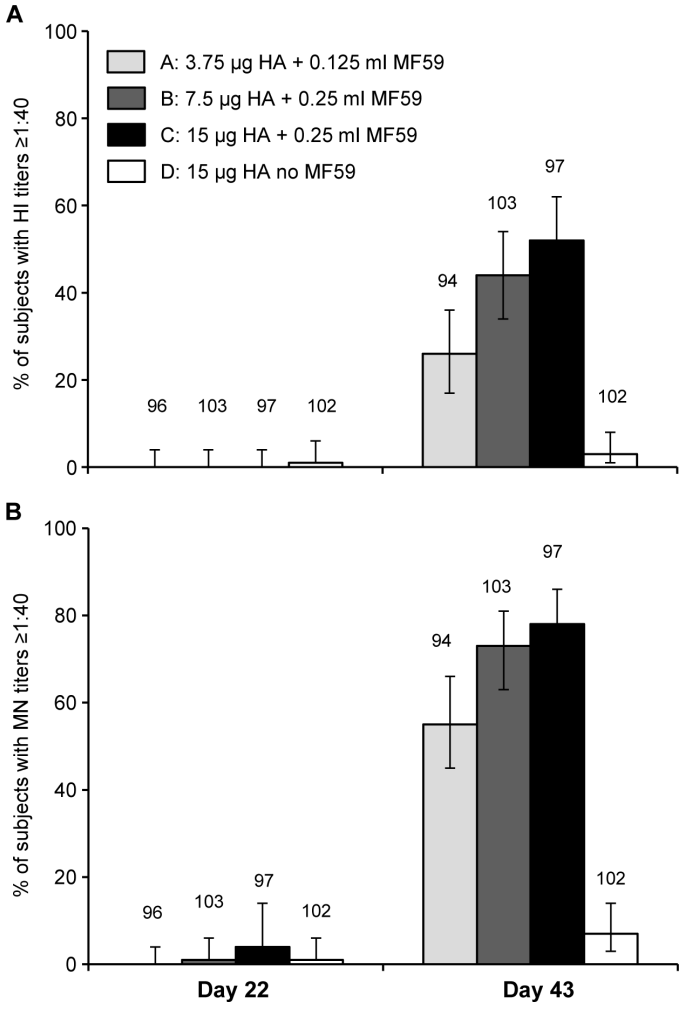

Fig. 2. Percentage of subjects with HI (A) or MN (B) titers greater than or equal to 1:40 on day 22 (3 weeks after the first dose) and day 43 (3 weeks after the second dose) for each group. Corresponding 95% confidence intervals (CIs) are displayed, and the number of subjects analyzed per group is indicated above each column.

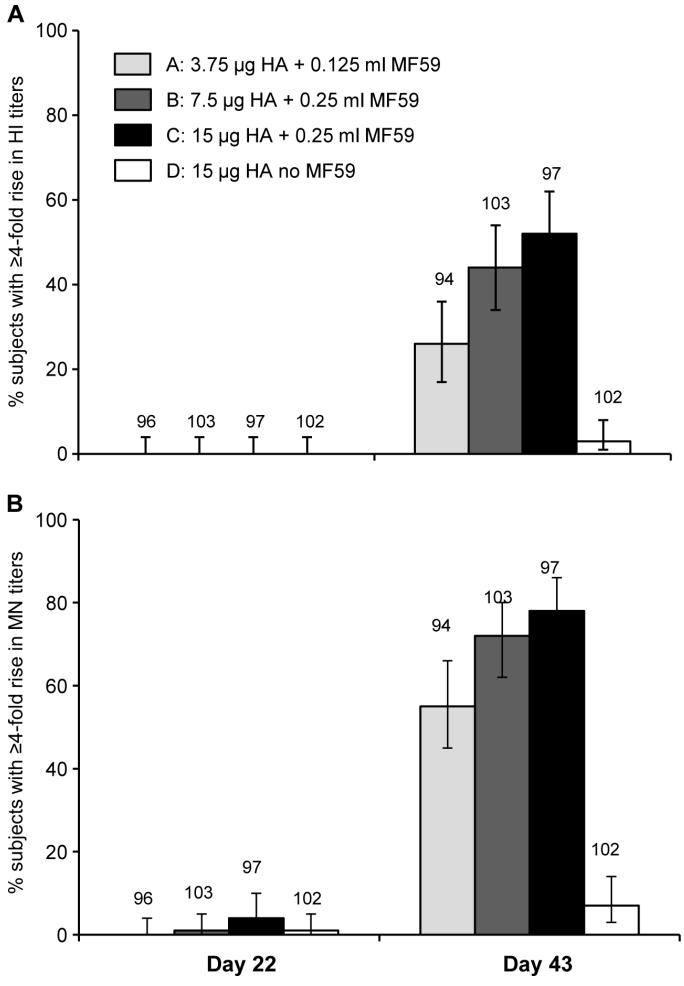

Fig. 3. Percentage of subjects exhibiting a fourfold or greater increase in HI (A) or MN (B) titers on day 22 (3 weeks after the first dose) and day 43 (3 weeks after the second dose) for each group. Corresponding 95% CIs are displayed, and the number of subjects analyzed per group is indicated above each column.

solicited local AEs in the MF59-adjuvanted groups, which predominantly consisted of mild injection site pain. The most common solicited systemic reactions were headache and fatigue. Group B reported the highest rates of solicited local and systemic effects among all groups. Overall, there were fewer AEs after the second vaccination than after the first. Severe solicited AEs were rare (<1%) and not aggregated in any specific treatment group. A total of 29% of subjects reported unsolicited AEs during days 1 to 43, of which 8% were considered possibly related to the study vaccination (see Table 3 for details). Unsolicited AEs primarily fell into the following categories: (i) "infections and infestations" (13%), mostly upper respiratory infection (4%) and nasopharyngitis (3%); (ii) "general disorders and administrative site conditions" (6%), primarily related to solicited local reactions; (iii) "musculoskeletal and connective tissue disorders" (5%), primarily arthralgia; and (iv) "nervous system disorders" (5%), with most reports being headache (4%). Unsolicited AEs occurred to the same degree across all groups. Two serious AEs were reported and were considered unrelated to the study vaccine: one subject was hospitalized for hip pain on day 23, and one subject was hospitalized with multiple bone fractures on day 36.

Serum chemistry and hematology clinical laboratory tests were also conducted and did not raise safety concerns. All average hematology values fell within the normal range. In addition, biochemical data collected on day 43 were, in general, similar to those collected on day 1. One subject, however, was reported to have elevated alanine aminotransferase on day 43 relative to day 1, which, in the absence of any other obvious explanation, was considered possibly related to the vaccine.

DISCUSSION

Given the urgent public health demand for widespread availability of vaccines in the face of a potential A/H7N9 influenza pandemic, rapid development of an effective and acceptable H7N9 vaccine is essential. Here, we thus present findings on the antigen-sparing

Table 3. Subjects with solicited adverse reactions. Maximum reaction severity reported from day 1 (first 30 min) to day 7 after vaccinations 1 and 2.

	Vaccination 1					Vaccination 2				
	Group A (*n* = 98)	Group B (*n* = 103)	Group C (*n* = 98)	Group D (*n* = 102)	Total (*n* = 401)	Group A (*n* = 95)	Group B (*n* = 102)	Group C (*n* = 97)	Group D (*n* = 101)	Total (*n* = 396)
Local reactions [*n* (%)*]										
Pain										
Any	42 (43)	74 (72)	53 (54)	19 (19)	188 (47)	34 (36)	53 (52)	45 (46)	21/100 (21)	153 (39)
Severe	0	0	0	0	0	1 (1)	0	0	0	1 (<1)
Ecchymosis (mm)										
Any	2 (2)	2 (2)	2 (2)	5 (5)	11 (3)	1 (1)	3 (3)	0	4 (4)	8 (2)
>100	0	0	0	0	0	0	0	0	0	0
Erythema (mm)										
Any	11 (11)	16 (16)	13 (13)	11 (11)	51 (13)	11 (12)	12 (12)	6 (6)	9 (9)	38 (10)
>100	0	0	0	0	0	0	0	0	0	0
Induration (mm)										
Any	5 (5)	9 (9)	6 (6)	4 (4)	24 (6)	9 (9)	16 (16)	4 (4)	6 (6)	35 (9)
>100	0	0	0	0	0	0	0	0	0	0
Systemic reactions [*n* (%)]										
Nausea										
Any	11 (11)	12 (12)	8 (8)	5 (5)	36 (9)	10 (11)	9 (9)	9 (9)	7 (7)	35 (9)
Severe	0	0	1 (1)	0	1 (<1)	0	0	1 (1)	0	1 (<1)
Myalgia										
Any	10 (10)	23 (22)	13 (13)	11 (11)	57 (14)	8 (8)	16 (16)	14 (14)	7 (7)	45 (11)
Severe	0	0	0	0	0	0	0	0	0	0
Arthralgia										
Any	4 (4)	13 (13)	10 (10)	8 (8)	35 (9)	5 (5)	12 (12)	12 (12)	3 (3)	32 (8)
Severe	0	0	0	0	0	0	0	0	0	0
Headache										
Any	23 (23)	36 (35)	23 (23)	20 (20)	102 (25)	18 (19)	19 (19)	17 (18)	11 (11)	65 (16)
Severe	0	1 (1)	0	1 (1)	2 (<1)	0	1 (1)	2 (2)	0	3 (1)
Fatigue										
Any	20 (20)	32 (31)	24 (24)	22 (22)	98 (24)	13 (14)	19 (19)	15 (15)	15 (15)	62 (16)
Severe	0	0	2 (2)	0	2 (<1)	0	3 (3)	0	0	3 (1)
Vomiting										
Any	3 (3)	2 (2)	2 (2)	0	7 (2)	1 (1)	0	1 (1)	1 (1)	3 (1)
Severe	0	0	0	0	0	0	0	1 (1)	0	1 (<1)
Diarrhea										
Any	10 (10)	9 (9)	6 (6)	7 (7)	32 (8)	5 (5)	6 (6)	4/95 (4)	8 (8)	23 (6)
Severe	0	0	1 (1)	1 (1)	2 (1)	0	0	0	1 (1)	1 (<1)
Loss of appetite										
Any	4 (4)	10 (10)	11 (11)	4 (4)	29 (7)	4 (4)	10 (10)	7 (7)	6 (6)	27 (7)
Severe	0	0	1 (1)	0	1 (<1)	0	0	0	0	0
Malaise										
Any	8 (8)	25 (24)	23 (23)	15 (15)	71 (18)	8 (8)	17 (17)	14 (14)	9 (9)	48 (12)
Severe	0	0	1 (1)	0	1 (<1)	0	2 (2)	1 (1)	0	3 (1)
Body temperature (≥38°C)										
Yes	0	1 (1)	1 (1)	1 (1)	3 (1)	0	2 (2)	0	0	2 (1)
Other reactions [*n* (%)]										
Temperature (°C)†										
36–36.4	16 (16)	13 (13)	10 (10)	10 (10)	49 (12)	15 (16)	11 (11)	4 (4)	14 (14)	44 (11)
≥40	0	0	1 (1)‡	0	1 (<1)‡	0	0	0	0	0
Prevention of pain and/or fever										
Yes	3/95 (3)	3 (3)	0	1 (1)	7 (2)	0	1 (1)	0	1 (1)	2 (1)
Treatment of pain and/or fever										
Yes	4 (4)	12 (12)	3 (3)	3 (3)	22 (5)	3 (3)	7 (7)	7 (7)	4 (4)	21 (5)

*Threshold for ecchymosis, erythema, and induration: none (0 mm), any (≥1 mm). †Irrespective of route of measurement. ‡Incorrect (implausible) value of 977°F was entered in the diary card by the subject, corresponding to 525°C, decimal error.

and immune-enhancing effects of MF59, an oil-in-water emulsion adjuvant already licensed for human use (*13*), on a candidate H7N9 vaccine. In this analysis, we demonstrate that the use of a synthetic virus–derived, mammalian cell culture–produced, inactivated subunit monovalent H7N9 pandemic influenza vaccine, adjuvanted with MF59, results in effective immune responses in immunologically naïve subjects after two doses. These responses are comparable to those observed in convalescent sera after H7N9 infection—MN range 20 to 80, GMT 40 and HI range 20 to 640, GMT 118 (*24*)—and exceed those observed with previous H7 vaccines (*5*).

At the time of analysis (day 43, 3 weeks after the second dose), a dose-dependent trend was observed, with highest responses occurring in the group administered the MF59-adjuvanted 15-μg HA formulation (Figs. 2 and 3). In this cohort, 52 and 78% of subjects exhibited titers of ≥1:40 in the HI (Fig. 2A) and MN (Fig. 2B) assays, respectively. The poorest immune responses were seen in the nonadjuvanted 15-μg HA group. Overall, our findings are consistent with results from previous clinical studies comparing MF59-adjuvanted and nonadjuvanted seasonal and pandemic influenza vaccines (*25*–*29*). Moreover, our results are also consistent with a recent trial of an investigational saponin-based ISCOMATRIX-adjuvanted H7N9 vaccine (*30*), which likewise showed improved immune responses in the group receiving the adjuvanted vaccine compared to the group receiving nonadjuvanted vaccine with higher HA content.

In this analysis, we used both the HI and MN assays to assess response. Although both assays showed dose-response effects in the MF59-adjuvanted groups by day 43 (Figs. 2 and 3), the observed MN values were consistently higher than the HI values. This was expected because we and others have previously observed a similar trend toward higher MN titers compared to HI titers after administration of either H5N1 or H9N2 pandemic influenza vaccines to human subjects (*11*, *21*, *23*, *31*, *32*). Because the MN assay detects not only the antibodies that interfere with the receptor binding domain of viral HA but also other functional antibodies (*33*), it has been suggested that the MN assay is a more sensitive approach to assessing antibody responses to avian influenza strains. Indeed, after infection of experimental animals with avian influenza strains, neutralizing antibody responses, detected by the MN assay, but not corresponding HI responses, have been associated with protection from rechallenge with homologous strains (*34*). Moreover, a clinical article recently reported that early and rapid induction of MN antibodies, but not HI titers, in patients infected with H7N9 correlated with rapid recovery from illness (*35*). Hence, the MN assay may represent a more accurate predictor of protection from infection by these viruses. In the absence of a proven correlate of protection for A/H7N9 infection, however, the results from both MN and HI assays need to be considered together when assessing immunological responses to A/H7N9 vaccines (*36*).

Immunogenicity effects with the MF59-adjuvanted H7N9c formulations were first detected on day 43, 3 weeks after the second dose. At day 22, 3 weeks after the first dose, only one subject (in group D) had an HI titer of ≥1:40 and no subjects exhibited a fourfold or greater rise in HI titer over baseline (Figs. 2A and 3A). Analysis of the MN results likewise showed minimal responses on day 22, with only 1, 4, and 1% of subjects in groups B, C, and D, respectively (Figs. 2B and 3B), reaching titers of ≥1:40. This observation that two doses are required for effective immunogenicity is similar to previous clinical reports on adjuvanted pandemic avian influenza vaccines (*20*, *21*, *32*, *37*–*39*) as well as to a recent report on another investigative A/H7N9 vaccine (*30*). Moreover, a very recent report that examined the antibody responses to the A/H7N9 virus infection likewise confirmed that the natural immune response to this influenza strain is weak, and consequently, multiple vaccinations may be required to achieve protective immunity (*24*). Collectively, these studies suggest that at least two doses of an H7N9 vaccine will be required to induce an effective immune response in immunologically naïve individuals. It is thus anticipated that a two-dose regimen in adults, and probably other target age groups, will be ultimately necessary to prevent symptoms and to reduce A/H7N9 transmission.

One consideration to bear in mind is that although the A/H7N9 virus can infect humans of all ages, elderly subjects appear to develop more severe disease. Here, the mean age of subjects ranged from 38.3 to 43.4 years. On the basis of previous experience with other cell culture–derived H5N1 and H1N1 vaccines [for example, (*40*)], which have shown that elderly subjects have similar but lower immune responses compared to adult subjects, it is expected that an analogous pattern would be seen with the H7N9 vaccine, necessitating two doses of adjuvanted vaccine.

In the rational design of a feasible adjuvanted H7N9 vaccine, MF59 is a natural adjuvant of choice, given that it is already used in vaccines licensed in many countries and is well characterized with more than 16 years of safety data from clinical trials and postmarketing pharmacovigilance (*13*, *16*). In addition, the MF59-adjuvanted H7N9c vaccine formulations (containing 3.75, 7.5, or 15 μg of HA antigen) used in this phase 1 study have comparable dose-sparing immunogenicity to other MF59-adjuvanted licensed candidate pandemic vaccines (*14*, *23*, *32*, *40*–*43*). In these clinical trials, MF59-adjuvanted formulations resulted in a clear increase in immunogenicity with much lower antigen content compared to nonadjuvanted formulations. Moreover, like the MF59-adjuvanted preparations in the present study, two doses were required to achieve appropriate levels of serologic response in immunologically naïve populations. Another advantage with using MF59 is that it has been shown to induce both higher and broader antibody responses compared to other adjuvants such as aluminum (*27*). Consequently, the addition of an MF59 adjuvant could potentially expand the antibody repertoire to other antigenically drifted influenza variants.

In addition to developing antigen-sparing formulations, the ability to generate vaccines rapidly is paramount during a pandemic. Unfortunately, previous attempts to respond to impending influenza pandemics have thus far failed to be rapid or robust enough to preempt their occurrence (*44*). This is largely attributable to the slow process used for the conventional manufacture of influenza vaccines, which involves bulk growth of the virus in embryonated chicken eggs. This approach to vaccine production can take many months, by which time the peak of a pandemic may be over. A much more streamlined procedure has been recently developed (*8*), in which the genes for the two influenza proteins (HA and NA) needed for vaccine production are synthetically produced on the basis of electronically transmitted sequence data, and transfected into MDCK cells, along with sequences coding for other important viral genes. This synthetic seed approach for virus production has been shown to yield rapid and robust quantities of vaccine antigen (*8*). Notably, inoculating ferrets with a synthetic H7N9 virus, generated using this method, elicited a comparable immune response to inoculation with a nonsynthetic viral isolate (*8*). Here, we used similar synthetic and mammalian cell culture technology to generate our inactivated A/H7N9 influenza pandemic vaccine. In doing so, we show that we have the potential to meet the demand for an A/H7N9 vaccine, should the need arise.

Administration of the synthetic seed/MDCK cell–derived vaccine was well tolerated in both MF59-adjuvanted and nonadjuvanted groups with minimal safety concerns. The incidence of AEs that could be associated with the cell culture–derived vaccine was consistent with previous reports on the safety of a cell culture–derived H5N1 vaccine (*11*) and was comparable to that reported for egg-derived inactivated influenza vaccines (*38*, *45*). As previously observed (*46*, *47*), the addition of MF59 was associated with an increased incidence of injection site pain and other general effects (such as headache and fatigue), but these were typically mild and short-lived. The number of unsolicited adverse events was generally low and occurred in all treatment groups to the same degree.

Although this study indicates that our candidate MF59-adjuvanted vaccine may meet the requirements of a pandemic H7N9 vaccine, there are a number of limitations to consider. For example, although reaching an HI titer of 40 is widely regarded as the standard criterion for immunological protection against influenza infection in adult populations (Center for Biologics Evaluation and Research), this correlate of protection has not been established for all cases. Thus, for different age cohorts, populations, and influenza strains, correlating HI responses with other immunological assays is necessary (*48*). Accordingly, for the novel A/H7N9 viral strain, it remains uncertain what level of HI titer confers protection against illness. However, in a relatively recent report, H7 vaccines were shown to have a protective effect in an animal model of influenza, despite no or low HI titers (*49*). Such observations imply that other mechanisms are involved in influenza protection that could be probed for novel markers of influenza vaccine efficacy. For now, in the absence of alternative markers, our analysis has remained focused only on established HI and MN measures. Another limitation of the study is that, because the focus was on developing a dose-sparing vaccine formulation, higher nonadjuvanted and MF59-adjuvanted doses of the H7N9c vaccine were not examined. Whereas higher nonadjuvanted doses of H1N1 vaccines have been reported to increase immune effects (*45*), higher doses of nonadjuvanted H5N1 vaccines have not shown encouraging responses (*46*). Similarly, the recent analysis of an investigational A/H7N9 vaccine indicated that higher nonadjuvanted doses (up to 45 μg) had minimal effects on immunogenicity (*30*). Thus, on the basis of these studies, higher nonadjuvanted doses of our H7N9c vaccine would not be expected to show increased immunogenicity.

Here, we examined MF59-adjuvanted H7N9c vaccine formulations, containing varying antigen and adjuvant doses, to assess whether these formulations elicit effective immune responses against the A/H7N9 virus. Given the small size of the vaccine cohorts, however, this study was not powered to conduct immunological comparisons between groups. Nonetheless, the results of these studies have implications for the rational design (amount of antigen and adjuvant per dose) of an H7N9c vaccine intended for immunologically naïve adults. Ultimately, the key findings of this phase 1 study are that an effective H7N9 vaccine is achievable and that by adding the established adjuvant MF59 to the formulation, robust antibody responses can be attained in most individuals after a second injection. MF59 has the benefit of not only improving the effect of the vaccine but also sparing the use of antigen. Furthermore, by using cell culture technology to produce the virus from a synthetic seed, vaccine production can be expedited and scaled up to ensure that we meet the demands of a possible pandemic. The results of this phase 1 trial represent a significant advance toward achieving a credible vaccine against the A/H7N9 virus outbreak, which has claimed 115 lives to date.

MATERIALS AND METHODS

Study design

This phase 1, multicenter, observer-blind, dose-ranging study was initiated in August 2013 at six sites in the United States (ClinicalTrials.gov identifier: NCT 01928472). Healthy adult subjects aged 18 to <65 years were equally randomized to one of four vaccine groups, at about 100 subjects per group. Each subject received two H7N9c vaccine doses, administered 3 weeks apart. Blood samples for immunoassays (HI and MN) were collected from all subjects before any vaccination (days 1 and 22) as well as at clinical visits on days 43, 183, and 366. Here, subjects were monitored up until day 43 for immunogenicity and safety. Follow-up of antibody persistence and safety from days 43 to 366 will be published at a later date.

The study protocol was approved by the Ethics Review Committees/Institutional Review Boards for each site and was designed in accordance with the Declaration of Helsinki, the U.S. Department of Health and Human Services guidelines, and the principles of Good Clinical Practice. Written informed consent was obtained from all participants before enrollment.

The primary objective of the study was to evaluate the antibody response to different doses of MF59-adjuvanted and nonadjuvanted H7N9c vaccine (3.75, 7.5, or 15 μg) after the second dose (day 43) using the HI assay. The secondary objectives were to assess antibody responses after a single vaccine dose (day 22) and the level of antibody responses measured by the MN assay. Immunogenicity results up to day 43 are presented.

Assuming a 10% dropout rate, a sample size of 90 evaluable subjects per vaccine group was predicted to have 80% power to detect a 2.6-fold difference in GMT between two vaccine groups, assuming that the common SD of $\log_{10}$-transformed titer was 1.0 and using a two-group *t* test with a two-sided significance level of 0.05. With SDs of 0.8 and 0.6, the detectable difference could be further decreased 2.2- and 1.8-fold, respectively.

Subjects

Four hundred two subjects of ages between 18 and 64 years were enrolled and randomized into one of four vaccine dose groups in a 1:1:1:1 ratio, using a Web-based randomization system. Each cohort received two immunizations with the indicated vaccine formulations, 3 weeks apart. Subjects were excluded if any of the following applied: cognitive impairment interfering with the subject's ability to participate in the study; a progressive or severe neurologic disorder; a seizure disorder or history of Guillain-Barré syndrome; known or suspected immune system impairment; pregnancy or breast-feeding; women of child-bearing age unwilling to use birth control methods from 2 months before the study until 3 weeks after the last vaccination; allergy to the vaccine components; a malignancy or lymphoproliferative disorder within the past 5 years; participation in another clinical trial from 30 days before, and up until the end of, the study; body temperature ≥38°C or any acute illness within 3 days of the study vaccination; previous suspected or confirmed H7N9 illness; previous receipt of any H7 vaccine; previous receipt of any influenza vaccine within 14 days before enrollment or intended receipt before day 44 in the study; receipt of any inactivated vaccine within 2 weeks or any live vaccine within 4 weeks of the start of the study; research staff or their families/household members; body mass index >35 kg/m^2; history of drug or alcohol abuse in the past 2 years.

Vaccines

All H7N9c vaccine formulations included purified surface antigens, HA and NA, from the A/Shanghai/2/2013 H7N9c influenza strain that were generated from synthetic seed virus propagated in MDCK cells, essentially as described previously (*8*). In collaboration with Synthetic Genomics Vaccine Inc., these genes were synthesized and combined with PR8x backbone genes to produce a vaccine virus. This virus then underwent limiting dilution subcloning at Philipps-Universität Marburg before being used to seed vaccine manufacturing in MDCK cells. The HA content of the vaccine was determined by reversed-phase high pressure liquid chromatography. For formulations containing MF59 adjuvant, vaccines were prepared by extemporaneous mixing, immediately before administration. To obtain the indicated vaccine compositions, appropriate volumes of H7N9c HA (supplied as a prefilled vial of 15 μg/0.5 ml, with a total vial content of 0.6 ml) and MF59 adjuvant (9.75 mg/0.25 ml, with a total vial content of 0.7 ml) were combined. Subjects in the MF59-adjuvanted groups thus received two doses of the following: group A, 0.25-ml injected volume containing 3.75 μg of HA + 0.125 ml (containing 4.875 mg of squalene) of MF59; group B, 0.5-ml injected volume containing 7.5 μg of HA + 0.25 ml (containing 9.75 mg of squalene) of MF59; and group C, 0.75-ml injected volume containing 15 μg of HA + 0.25 ml (containing 9.75 mg of squalene) of MF59. In these groups, MF59 was administered as either a half (0.125-ml) or full (0.25-ml) dose in relation to the MF59 dose included in the already approved MF59-adjuvanted vaccine Fluad. Subjects in group D received a prefilled 0.5-ml dose containing 15 μg of HA without the MF59 adjuvant. Vaccines were administered intramuscularly into the deltoid muscle of the nondominant arm.

Immunogenicity

A minimum blood volume of 10 ml was drawn from each subject at every visit (days 1, 22, and 43) and before any vaccination. Antibody responses were evaluated by performing standard HI and MN assay protocols using a homologous A/H7N9 vaccine strain (starting dilutions, 1:10). The HI assay was conducted using turkey erythrocytes and was based on a previously described method (*50*), and the MN assay was performed according to the method described by Rowe *et al.* (*51*). Results from both the HI and MN analyses are expressed as follows: (i) GMT, (ii) percentage of subjects with titers ≥1:40, and (iii) percentage of subjects with a fourfold or greater increase in postvaccination titer from baseline. Serological evaluations were conducted at the Novartis Vaccines Clinical Laboratory Sciences Department in Marburg, Germany, and all samples were tested by investigators blinded to subject identification, visit number, and treatment assignment.

Reactogenicity and safety

Subjects were observed for 30 min after each vaccination (vaccination 1: day 1; vaccination 2: day 22) to monitor for immediate adverse reactions. Each subject was then provided with a diary card to record local and systemic reactions for 7 days after each vaccination (that is, days 1 to 7 and days 22 to 28). Solicited local reactions were as follows: injection site induration, erythema, ecchymosis, and injection site pain. Solicited systemic reactions were as follows: nausea, myalgia, arthralgia, headache, fatigue, vomiting, diarrhea, loss of appetite, malaise, and fever (body temperature ≥38°C). Subjects were also asked to record any unsolicited adverse event (AE) during the treatment period (that is, up to and including day 43) including any serious AE. Solicited reactions and AEs were classed as mild (no limitation to normal daily activity), moderate (some limitation to normal daily activity), or severe (unable to perform normal activity) and were then classed as unrelated, possibly related, or probably related to the study vaccination. Clinical safety laboratory assessments were also performed on all subjects on days 1 and 43. Hematology data collected included the following: hematocrit (%), hemoglobin (g/liter), erythrocyte mean corpuscular hemoglobin (pg/cell), erythrocyte mean corpuscular hemoglobin concentration (g/liter), erythrocyte mean corpuscular hemoglobin volume (fl), platelets ($\times 10^9$/liter), erythrocytes ($\times 10^{12}$/liter), and leukocytes ($\times 10^9$/liter). Biochemistry data collected included alanine aminotransferase (IU/liter), aspartate aminotransferase (IU/liter), and creatinine (μM).

Statistical analyses

No formal statistical hypotheses were tested for the immunogenicity and safety data. HI and MN assay results were analyzed descriptively using point estimates by vaccine group and two-sided 95% CIs, estimated according to the Clopper-Pearson method. GMTs and their associated 95% CIs were determined for each vaccine group using unadjusted estimation.

All immunogenicity analyses were performed on the full analysis set, that is, on subjects who had received a study vaccination and who had provided evaluable sera. The percentage of subjects achieving an HI titer of ≥1:40 and the percentage of subjects exhibiting a fourfold or greater increase in postvaccination titer from baseline (along with the 95% CIs) were determined using an exact method of Clopper-Pearson.

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