

ORIGINAL ARTICLE

# A Novel Influenza A (H1N1) Vaccine in Various Age Groups

Feng-Cai Zhu, M.D., Hua Wang, M.D., Han-Hua Fang, M.D., Jian Guo Yang, M.D., Xiao Jun Lin, M.D., Xiao-Feng Liang, M.D., Xue-Feng Zhang, M.D., Hong-Xing Pan, M.D., Fan-Yue Meng, M.D., Yue Mei Hu, M.D., Wen-Dong Liu, M.D., Chang-Gui Li, M.D., Wei Li, M.D., Xiang Zhang, M.D., Jin Mei Hu, M.D., Wei Bing Peng, M.D., Bao Ping Yang, M.D., Pei Xi, M.D., Hua-Qing Wang, M.D., and Jing-Shan Zheng, M.D.\*

## ABSTRACT

### BACKGROUND

There is an urgent need for a vaccine that is effective against the 2009 pandemic influenza A (H1N1) virus.

### METHODS

A split-virus, inactivated candidate vaccine against the 2009 H1N1 virus was manufactured, and we evaluated its safety and immunogenicity in a randomized clinical trial. Subjects were between 3 and 77 years of age, stratified into four age groups. The immunization schedule consisted of two vaccinations, 21 days apart. Subjects were injected with placebo or with vaccine, with or without alum adjuvant, at doses of 7.5  $\mu\text{g}$ , 15  $\mu\text{g}$ , or 30  $\mu\text{g}$ . Serologic analysis was performed at baseline and on days 21 and 35.

### RESULTS

A total of 2200 subjects received one dose, and 2103 (95.6%) received the second dose, of vaccine or placebo. No severe adverse side effects associated with the vaccine were noted. In the nonadjuvanted-vaccine groups, injection-site or systemic reactions, most mild in nature, were noted in 5.5 to 15.9% of subjects. Among the subjects receiving 15  $\mu\text{g}$  of nonadjuvanted vaccine, a hemagglutination-inhibition titer of 1:40 or more was achieved by day 21 in 74.5% of subjects between 3 and 11 years of age, 97.1% of subjects between 12 and 17 years, 97.1% of subjects between 18 and 60 years, and 79.1% of subjects 61 years of age or older; by day 35, the titer had been achieved in 98.1%, 100%, 97.1%, and 93.3% of subjects, respectively. The proportion with a titer of 1:40 or more was generally highest among the subjects receiving 30  $\mu\text{g}$  of vaccine, with or without adjuvant. Vaccine without adjuvant was associated with fewer local reactions and greater immune responses than was vaccine with adjuvant.

### CONCLUSIONS

These data suggest that a single dose of 15  $\mu\text{g}$  of hemagglutinin antigen without alum adjuvant induces a typically protective immune response in the majority of subjects between 12 and 60 years of age. Lesser immune responses were seen after a single dose of vaccine in younger and older subjects. (ClinicalTrials.gov number, NCT00975572.)

From Jiangsu Provincial Center for Disease Control and Prevention, Nanjing (F.-C.Z., H.W., X.-F.Z., H.-X.P., F.-Y.M., Y.M.H., W.-D.L.); National Institute for the Control of Pharmaceuticals and Biological Products, Beijing (H.-H.F., C.-G.L., W.L., X.Z.); Taizhou Municipal Center for Disease Control and Prevention, Taizhou (J.G.Y., X.Z., J.M.H., W.B.P.); Hualan Biological Bacterin Company, Xinxiang (X.J.L., B.P.Y., P.X.); and Chinese Center for Disease Control and Prevention, Beijing (X.-F.L., H.-Q.W., J.-S.Z.) — all in China. Address reprint requests to Dr. Zhu at the Center for Vaccine Clinical Trials, Jiangsu Provincial Center for Disease Control and Prevention, 172 Jiangsu Rd., Nanjing 210009, China, or at jszfc@vip.sina.com.

\*Other study investigators are listed in the Appendix.

This article (10.1056/NEJMoa0908535) was published on October 21, 2009, at NEJM.org.

N Engl J Med 2009;361.  
Copyright © 2009 Massachusetts Medical Society.

RECENTLY, A NOVEL SWINE-ORIGIN INFLUENZA A (H1N1) virus was identified as the cause of large numbers of febrile respiratory illnesses in Mexico and the United States.<sup>1,2</sup> It rapidly spread to many countries around the world, prompting the World Health Organization to declare a pandemic on June 11, 2009.<sup>3</sup> An important method of controlling this pandemic will be large-scale immunization. Currently used trivalent seasonal-influenza vaccines are unlikely to provide protection against the new 2009 pandemic influenza A (H1N1) virus<sup>4</sup>; hence, there is an urgent need to develop a vaccine against it. On July 29, 2009, the Advisory Committee on Immunization Practices tentatively concluded that children under 9 years of age may need to be given two doses of vaccine to elicit sufficient immunogenic reactivity against the 2009 H1N1 virus.<sup>5</sup>

In response to the pandemic, a novel vaccine against the virus strain A/California/07/2009 (H1N1) has been developed and recently was approved for sale in China. This report details the findings of a randomized, placebo-controlled, double-blind clinical trial of the safety and immunogenicity profile of this influenza A (H1N1) 2009 monovalent vaccine. In addition, we evaluated the role of alum adjuvant in the vaccine formulation, the optimal amount of antigen, and the need for second doses in children or elderly people.

## METHODS

### STUDY DESIGN AND OBJECTIVE

From July 2009 through August 2009, we enrolled a total of 2200 subjects between the ages of 3 years and 77 years in a stratified, randomized, double-blind, placebo-controlled, clinical trial in Taizhou, Jiangsu Province, China. The study was sponsored by Hualan Biological Bacterin Company. The study was conducted and the data were gathered by the nonindustry investigators, and the data analysis was conducted by Southeast University; all the authors drafted the manuscript and made the decision to submit it for publication. The sponsor did not influence these activities. All the authors had full access to the data, which were held by Southeast University, and all vouch for the accuracy and completeness of the data and the analysis.

Approval for the study protocol was obtained

from the ethics committee of the Jiangsu Provincial Center for Disease Control and Prevention, and the study was conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (as defined by the International Conference on Harmonization), and Chinese regulatory requirements, as stipulated by the Chinese Food and Drug Administration. Written informed consent was obtained from each subject or his or her legal representative.

The study subjects received either vaccine or placebo. The vaccine was administered at various doses, with or without alum adjuvant. The primary immunologic end points were the proportions of subjects with an increase in the hemagglutination-inhibition titer by a factor of 4 or more on day 21 after the first dose and on day 35 (14 days after the second dose). The second dose was administered on day 21, after data for the primary end point had been collected. The primary safety end points were the presence of any systemic reaction or injection-site reaction 21 days after the first dose and 14 days after the second dose.

### VACCINE

The influenza A (H1N1) 2009 monovalent, split-virus vaccine was developed by Hualan Biological Bacterin Company, and the seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College, New York), distributed by the Centers for Disease Control and Prevention in the United States. This strain was recommended by the World Health Organization and obtained from the Chinese Food and Drug Administration.<sup>6</sup> The vaccine was prepared in embryonated chicken eggs according to the same standard techniques that are used for the production of trivalent inactivated vaccine against seasonal influenza. In brief, the virus was harvested from the egg cultures and inactivated with the use of formaldehyde. The virus was concentrated, purified, and further sterilized by means of chromatography (on a Sepharose 4FF column) with Triton X-100.

The five experimental vaccines produced were split-virus products of 30  $\mu$ g of hemagglutinin antigen with or without adjuvant, 15  $\mu$ g of hemagglutinin antigen with or without adjuvant, and 7.5  $\mu$ g of hemagglutinin antigen with adjuvant. The adjuvant formulation contained 1.2 mg

of alum per milliliter of solution. The placebo consisted of phosphate-buffered saline.

#### STUDY SUBJECTS

Subjects were eligible to participate in the study if they were healthy, were 3 years of age or older, did not have a history of infection with the 2009 H1N1 virus, and had not received an influenza A (H1N1) 2009 monovalent vaccine, and if they or their guardians confirmed that they understood the study procedures, provided written informed consent, and agreed to keep a daily record of symptoms. Adverse events were coded according to the requirements of the Chinese Food and Drug Administration. These requirements are based on documents published by the Division of Microbiology and Infectious Diseases of the National Institutes of Allergy and Infectious Diseases that provide a grading system to classify adverse effects in adult and pediatric patients.<sup>7,8</sup> Women between 16 and 50 years of age were also required to have a negative pregnancy test at the time of screening and before each vaccination.

The study subjects were classified into four age categories: 61 years of age or older, 18 to 60 years of age, 12 to 17 years of age, and 3 to 11 years of age. The general rationale for the age divisions was as follows: young children, defined as those 3 to 11 years of age, attend kindergartens or primary schools; adolescents, defined as those 12 to 17 years of age, attend junior high school or high school; adults, defined as those 18 to 60 years, work outside the home; and the elderly, defined as those 61 years or older, are retired and often live with family. Hence, the four age groups have different socialization patterns and thus different risks of exposure to the virus.

Each treatment group had 110 subjects (Fig. 1). Subjects in the elderly and adolescent cohorts were each randomly assigned to receive one of five doses of vaccine: adjuvant vaccine with 7.5, 15, or 30  $\mu\text{g}$  of hemagglutinin antigen per dose or nonadjuvant vaccine with 15 or 30  $\mu\text{g}$  of hemagglutinin antigen per dose. The adult subjects were randomly assigned to receive placebo or one of five doses of vaccine: adjuvant vaccine with 7.5, 15, or 30  $\mu\text{g}$  of hemagglutinin antigen per dose or nonadjuvant vaccine with 15 or 30  $\mu\text{g}$  of hemagglutinin antigen per dose. The subjects who were 3 to 11 years of age were randomly assigned to receive one of four doses of

vaccine: adjuvant vaccine with 7.5 or 15  $\mu\text{g}$  of hemagglutinin antigen per dose or nonadjuvant vaccine with 15 or 30  $\mu\text{g}$  of hemagglutinin antigen per dose.

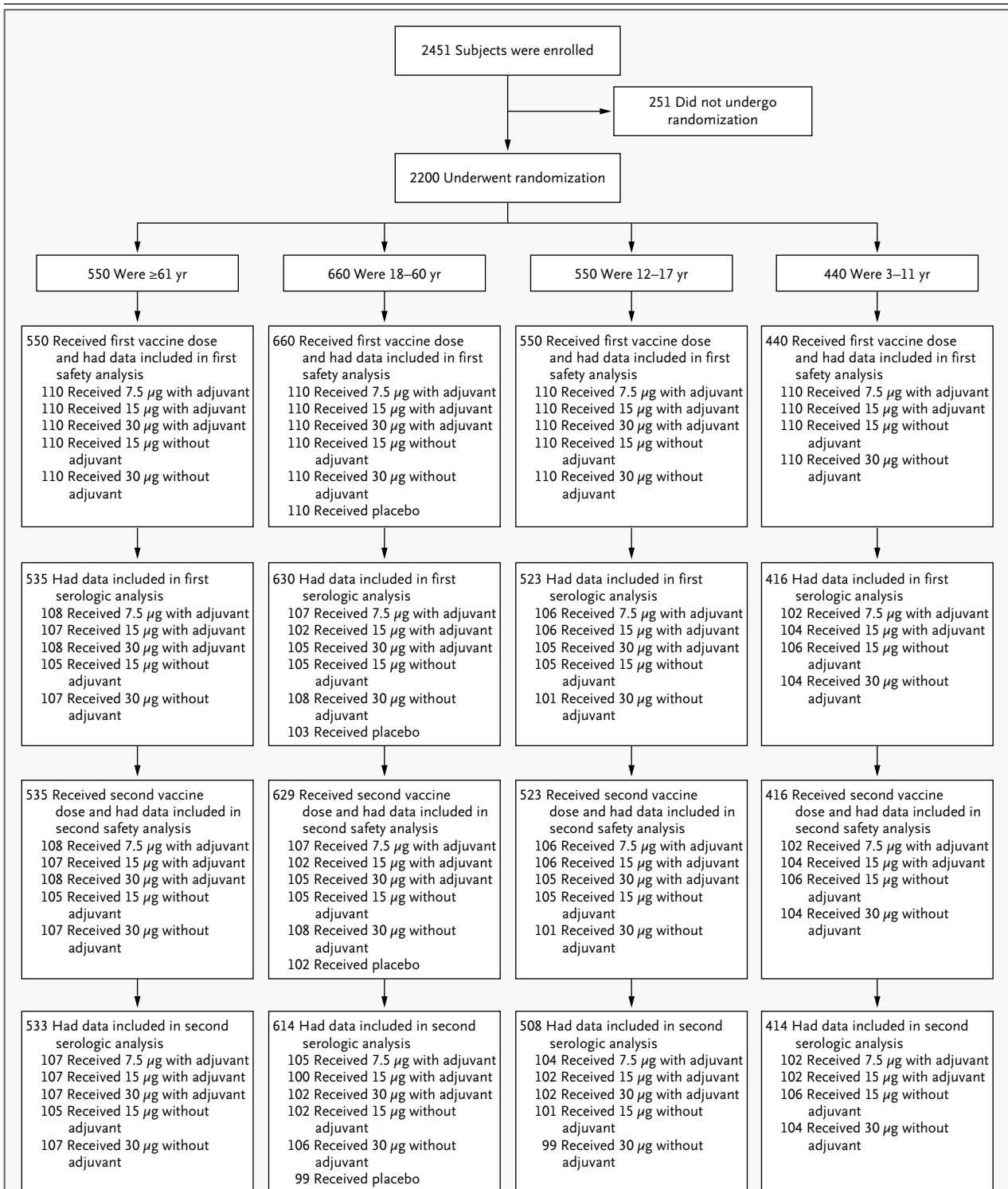
With 110 subjects per treatment group, the study had a statistical power of at least 80% to detect a seroconversion rate of more than 40% in each group. The randomization schedule was created with the use of SAS software (version 9.1). The block size was defined by an independent statistician at Southeast University (Nanjing, China). Randomization-code numbers were assigned to subjects in chronological order by the investigator.

Subjects and investigators were unaware of the formulation of the study vaccine that was administered. Injections were given intramuscularly, in the deltoid muscle. After an on-site safety observation of 30 minutes' duration, subjects or their guardians were asked to record underarm body temperature and data on injection-site reactions and systemic reactions at 6, 24, 48, and 72 hours and at 7, 14, and 21 days, in a diary provided by the investigators. Serum samples were collected three times: on day 0 (before the first vaccination), day 21 (before the second vaccination), and day 35 (14 days after the second vaccination).

#### ASSAYS

The titer of antibodies against the vaccine strain was measured in all samples by means of hemagglutination-inhibition assays, which were performed in accordance with established procedures and with the use of turkey erythrocytes.<sup>9</sup> In brief, samples were treated with cholera filtrate at 36°C for 16 hours and were then tested at dilutions of 1:10 and 1:20. Titers of anti-hemagglutinin antigen antibodies that were below the detection limit (i.e., <1:10) were assigned a value of 1:5, and titers above 1:5120 were assigned a value of 1:5120. All samples were assayed in a blinded manner, in duplicate, and were double-checked by two investigators at the Chinese National Institute for the Control of Pharmaceuticals and Biological Products.

The results of immunogenicity assays performed 21 days after the first vaccination and 14 days after the second vaccination were compared with the results obtained at baseline. In addition, the numbers of subjects who underwent seroconversion were noted and compared with



**Figure 1. Enrollment and Follow-up of the Study Subjects.**

The reasons why 251 subjects did not undergo randomization are listed in Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. The reasons why 97 of the 2200 subjects who received the first vaccine dose did not receive the second dose are listed in Table 2 in the Supplementary Appendix.

the baseline numbers. Seroconversion was defined as an increase in the hemagglutination-inhibition titer of at least four times the baseline titer, according to international guidelines used to evaluate influenza vaccines.<sup>10,11</sup> No microneutralization tests were performed.

#### STATISTICAL ANALYSIS

The likelihood-ratio chi-square test or Fisher's exact test was used to compare the number of subjects in each treatment group who had a local (injection-site) or systemic reaction within 21 days after vaccination.

The primary immunologic end points were the proportions of subjects with an increase in the hemagglutination-inhibition titer by a factor of 4 or more after the first and second vaccine doses. Specifically, seroconversion was considered to have occurred when the hemagglutination-inhibition titer was less than 1:10 before vaccination and was 1:40 or more after vaccination or the titer was 1:10 or more before vaccination and increased to at least four times the baseline level after vaccination. Other end points regarding hemagglutination inhibition included the geometric mean titer, the geometric mean increase (i.e., the ratio of the titer after vaccination to the titer before vaccination), and the proportion of subjects with a titer of 1:40 or more. Modeling of the geometric mean titers and geometric mean increases was conducted with the use of generalized linear models, which included the effects of adjuvant status, dose, and age. Logistic regression was used to model the rate of adverse reactions, the proportion of subjects with an increase in the hemagglutination-inhibition titer by a factor of 4 or more, and the proportion with a titer of 1:40 or more, according to adjuvant status, dose, and age. Safety data were summarized descriptively.

Hypothesis testing was conducted with the use of two-sided tests, with an alpha value of 0.05 considered to indicate statistical significance. All statistical analyses were performed by means of SAS software (version 9.1).

---

## RESULTS

---

#### STUDY SUBJECTS

A total of 2200 subjects between 3 and 77 years of age received the first dose of vaccine, and 2103 (95.6%) received the second dose, 21 days later

(Fig. 1, and Table 2 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Data from all vaccinated subjects were included in the safety analysis. Immunogenicity data on the effects of the first and second vaccinations were available for 2104 and 2069 subjects, respectively.

#### SAFETY

Injection-site and systemic reactions are shown in Table 1. Most reactions were mild; grade 3 reactions — corresponding to an inability to perform usual social and functional activities<sup>7,8</sup> — occurred in 0.0 to 1.8% of the study subjects (Table 1, and Tables 3, 4, and 5 in the Supplementary Appendix). Only one severe adverse event was reported, in a subject who received placebo: hospitalization for atrial fibrillation on the day after the first injection. Thus, no serious, vaccine-related adverse events or unexpected events were recorded. Analysis of the factors associated with the rate of local or systemic reactions suggested that the presence of adjuvant and receipt of the second dose were associated with a higher rate of local reactions ( $P < 0.001$  and  $P = 0.002$ , respectively) (Table 6 in the Supplementary Appendix). For systemic reactions, age was the only factor with significant effects on the rate: subjects 12 to 17 years of age had systemic reactions most frequently, followed by subjects 3 to 11 years of age, those 18 to 60 years of age, and those 61 years of age or older (Table 7 in the Supplementary Appendix). The most common injection-site reaction after vaccination was pain, which occurred in 10.5 to 26.7% of subjects; the most frequently reported systemic reaction was fever, which occurred in 11.5 to 18.0% of subjects (Table 8 in the Supplementary Appendix). The prevalence of an adverse event was similar among subjects with detectable hemagglutination-inhibition antibodies against the vaccine strain and in those without detectable antibodies.

#### IMMUNE RESPONSE

The proportion with a hemagglutination-inhibition titer of 1:40 or more was 1.1 to 6.0% at baseline in the four age groups (Table 2, and Table 9 in the Supplementary Appendix). Hemagglutination-inhibition antibodies against the vaccine strain were detected on day 21 after the first dose and also 14 days after the second dose (day 35). The proportion with a titer of 1:40 or more and

Dose and Reaction Intensity*	3–11 Yr (N=440)	12–17 Yr (N=550)	18–60 Yr (N=660)	≥61 Yr (N=550)	Total (N=2200)
	<i>percent of subjects (95% CI)</i>				
<b>Injection-site reaction at day 21 (after first dose)</b>					
15 µg					
Reaction of any intensity	10.9 (5.8–18.3)	2.7 (0.6–7.8)	9.1 (4.5–16.1)	0.9 (0.0–5.0)	5.9 (3.9–8.5)
Grade 3 reaction	0.0 (0.0–3.3)	0.0 (0.0–3.3)	1.8 (0.2–6.4)	0.0 (0.0–3.3)	0.5 (0.1–1.6)
30 µg					
Reaction of any intensity	6.4 (2.6–12.7)	6.4 (2.6–12.7)	6.4 (2.6–12.7)	2.7 (0.6–7.8)	5.5 (3.5–8.0)
Grade 3 reaction	1.8 (0.2–6.4)	0.0 (0.0–3.3)	1.8 (0.2–6.4)	0.9 (0.0–5.0)	1.1 (0.4–2.6)
Placebo					
Reaction of any intensity			2.7 (0.6–7.8)		2.7 (0.6–7.8)
Grade 3 reaction			0.0 (0.0–3.3)		0.0 (0.0–3.3)
<b>Systemic reaction at day 21 (after first dose)</b>					
15 µg					
Reaction of any intensity	13.6 (7.8–21.5)	19.1 (12.2–27.7)	10.9 (5.8–18.3)	4.6 (1.5–10.3)	12.0 (9.2–15.5)
Grade 3 reaction	0.0 (0.0–3.3)	0.0 (0.0–3.3)	0.0 (0.0–3.3)	0.0 (0.0–3.3)	0.0 (0.0–0.8)
30 µg					
Reaction of any intensity	25.5 (17.6–34.7)	17.3 (10.7–25.7)	10.0 (5.1–17.2)	10.9 (5.8–18.3)	15.9 (12.6–19.7)
Grade 3 reaction	1.8 (0.2–6.4)	0.0 (0.0–3.3)	0.0 (0.0–3.3)	0.9 (0.0–5.0)	0.7 (0.1–2.0)
Placebo					
Reaction of any intensity			11.8 (6.5–19.4)		11.8 (6.5–19.4)
Grade 3 reaction			0.9 (0.0–5.0)		0.9 (0.0–5.0)
<b>Injection-site reaction at day 35 (after second dose)</b>					
15 µg					
Reaction of any intensity	10.4 (5.3–17.8)	7.6 (3.4–14.5)	6.7 (2.7–13.3)	4.8 (1.6–10.8)	7.4 (5.1–10.3)
Grade 3 reaction	0.0 (0.0–3.4)	0.0 (0.0–3.5)	0.0 (0.0–3.5)	0.0 (0.0–3.5)	0.0 (0.0–0.9)
30 µg					
Reaction of any intensity	7.7 (3.4–14.6)	7.9 (3.5–15.0)	4.6 (1.5–10.5)	11.2 (5.9–18.8)	7.9 (5.5–10.9)
Grade 3 reaction	0.0 (0.0–3.5)	0.0 (0.0–3.6)	0.9 (0.0–5.1)	0.9 (0.0–5.1)	0.5 (0.1–1.7)
Placebo					
Reaction of any intensity			2.9 (0.6–8.4)		2.9 (0.6–8.4)
Grade 3 reaction			0.0 (0.0–3.6)		0.0 (0.0–3.6)
<b>Systemic reaction at day 35 (after second dose)</b>					
15 µg					
Reaction of any intensity	11.3 (6.0–18.9)	16.2 (9.7–24.7)	3.8 (1.1–9.5)	1.9 (0.2–6.7)	8.3 (5.9–11.4)
Grade 3 reaction	0.0 (0.0–3.4)	0.0 (0.0–3.5)	0.0 (0.0–3.5)	0.0 (0.0–3.5)	0.0 (0.0–0.9)
30 µg					
Reaction of any intensity	11.5 (6.1–19.3)	18.8 (11.7–27.8)	7.4 (3.3–14.1)	7.5 (3.3–14.2)	11.2 (8.3–14.6)
Grade 3 reaction	1.0 (0.0–5.2)	0.0 (0.0–3.6)	0.0 (0.0–3.4)	0.0 (0.0–3.4)	0.2 (0.0–1.3)
Placebo					
Reaction of any intensity			7.8 (3.5–14.9)		7.8 (3.5–14.9)
Grade 3 reaction			0.0 (0.0–3.6)		0.0 (0.0–3.6)

\* A grade 3 reaction was one that resulted in an inability to perform usual social and functional activities.

**Table 2. Hemagglutination-Inhibition Titer of 1:40 or More among Subjects Receiving Nonadjuvanted Vaccine, According to Age Group.**

Day and Dose	3–11 Yr	12–17 Yr	18–60 Yr		≥61 Yr	All
			<i>percent of subjects (95% CI)</i>			
Day 0	1.1 (0.4–2.6)	6.0 (4.2–8.3)	4.3 (2.8–6.3)		4.0 (2.5–6.0)	4.0 (3.2–4.9)
Day 21 (after first dose)						
15 µg	74.5 (65.1–82.5)	97.1 (91.9–99.4)	97.1 (91.9–99.4)		79.1 (70.0–86.4)	86.9 (83.3–90.0)
30 µg	82.7 (74.0–89.4)	97.0 (91.6–99.4)	92.6 (85.9–96.8)		84.1 (75.8–90.5)	89.1 (85.7–91.9)
Placebo			10.7 (5.5–18.3)			10.7 (5.5–18.3)
Day 35 (after second dose)						
15 µg	98.1 (93.4–99.8)	100 (96.4–100)	97.1 (91.6–99.4)		93.3 (86.8–97.3)	97.1 (95.0–98.5)
30 µg	98.1 (93.2–99.8)	100 (96.3–100)	98.1 (93.4–100)		96.3 (90.7–99.0)	98.1 (96.3–99.2)
Placebo			11.1 (5.7–19.0)			11.1 (5.7–19.0)

**Table 3. Geometric Mean Titer of Hemagglutination-Inhibition Antibodies among Subjects Receiving Nonadjuvanted Vaccine, According to Age Group.\***

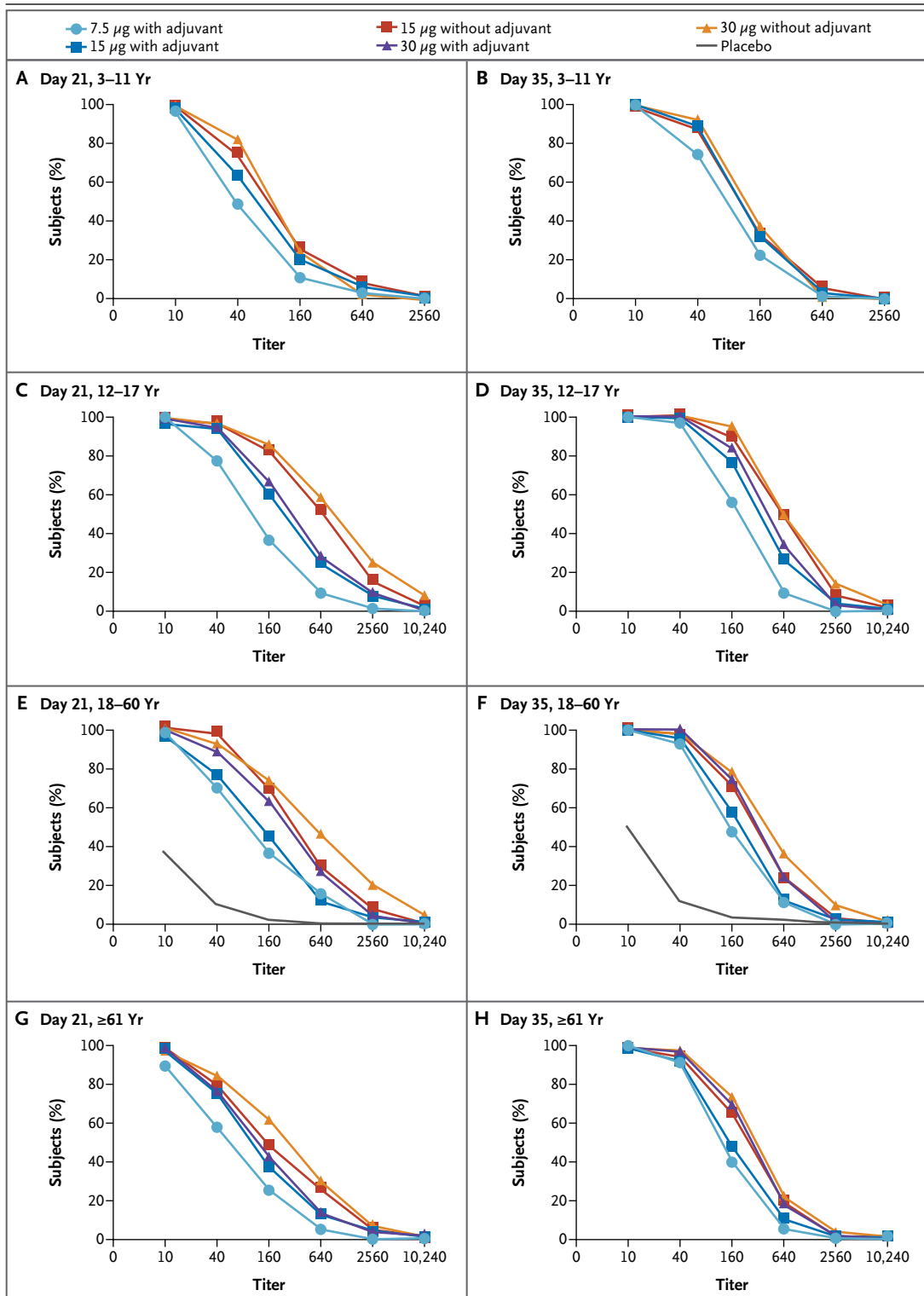
Day and Dose	3–11 Yr	12–17 Yr	18–60 Yr		≥61 Yr	All
			<i>geometric mean titer (95% CI)</i>			
Day 0	5.8 (5.6–6.0)	7.3 (6.9–7.8)	6.9 (6.5–7.2)		6.3 (6.0–6.7)	6.6 (6.4–6.8)
Day 21 (after first dose)						
15 µg	64.1 (51.1–80.3)	430.7 (330.0–562.1)	237.8 (184.3–306.8)		122.1 (88.8–167.8)	167.8 (144.6–194.8)
30 µg	66.4 (55.4–79.5)	680.8 (501.3–924.5)	370.9 (267.7–513.8)		178.6 (130.6–244.3)	232.7 (197.4–274.4)
Placebo			8.3 (7.0–9.8)			8.3 (7.0–9.8)
Day 35 (after second dose)						
15 µg	161.1 (134.0–193.5)	444.9 (356.6–555.0)	212.9 (173.9–260.5)		170.9 (135.6–215.4)	224.4 (200.9–250.7)
30 µg	165.4 (141.8–193.0)	518.8 (412.6–652.3)	311.7 (243.8–398.6)		210.0 (171.3–257.5)	271.3 (242.6–303.5)
Placebo			9.9 (8.1–12.0)			9.9 (8.1–12.0)

\* Anti-hemagglutinin antigen antibody titers below the detection limit (i.e., <1:10) were assigned a value of 1:5 for purposes of calculating the geometric mean titer.

the proportion with an increase in the hemagglutination-inhibition titer by a factor of 4 or more, according to treatment group, are listed in Table 10 in the Supplementary Appendix. Logistic-regression modeling showed that dose, adjuvant status, and age significantly affected the proportion of subjects with a titer of 1:40 or more and the proportion with an increase in the hemagglutination-inhibition titer by a factor of 4 or more after the first dose ( $P < 0.001$  for all comparisons) (Tables 11 and 12, respectively, in the Supplementary Appendix). Vaccine formulations without adjuvant were more immunogenic than formulations with adjuvant. There was also a dose-dependent antibody response; the dose of 30 µg was the most immunogenic, with the proportion of subjects with a titer of 1:40 or more highest among subjects 12 to 17 years of age, fol-

lowed by subjects 18 to 60 years of age, subjects 3 to 11 years of age, and subjects 61 years of age or older. The proportion of subjects with a titer of 1:40 or more among those receiving nonadjuvant vaccine was 74.5 to 97.1% after the first dose and increased to 93.3 to 100% after the second dose (Table 2).

The results were similar for the geometric mean titer and the geometric mean increase from baseline: the highest values in the adolescent and adult cohorts were seen with formulations without adjuvant and at higher doses (Tables 13, 14, and 15 in the Supplementary Appendix). Among the children, both the geometric mean titer and geometric mean increase rose significantly after the second vaccination. In contrast, in the other age groups, the antibody levels did not rise significantly after the second dose



**Figure 2.** Cumulative Distribution Curves for Titers of Hemagglutination-Inhibition Antibodies on Day 21 (after the First Dose of Vaccine) and Day 35 (after the Second Dose), According to Treatment Group and Age Group.

The percentages of subjects with hemagglutination-inhibition titers are shown for children (Panels A and B), adolescents (Panels C and D), adults (Panels E and F), and elderly subjects (Panels G and H). The limit of detection was a titer of 1:10. Titers are expressed as the reciprocal of the dilution.

among the subjects who received the nonadjuvanted vaccine. After the first and second vaccinations, antibody levels did not differ significantly between the groups receiving the 15- $\mu$ g dose of nonadjuvanted vaccine and the groups receiving the 30- $\mu$ g dose of nonadjuvanted vaccine (Table 3).

The antibody titers after the first and second doses of vaccine support the findings of higher immunogenicity of the vaccine formulations without adjuvant and of the higher doses, among adolescents and adults (Fig. 2).

---

## DISCUSSION

---

Recently, Greenberg et al.<sup>12</sup> reported that a single 15- $\mu$ g dose of split-virus 2009 H1N1 vaccine was immunogenic in healthy adults 18 to 64 years of age, with mild-to-moderate vaccine-associated reactions. However, because children and the elderly were not included in the study, it not known whether a second dose of 2009 H1N1 vaccine would be necessary to induce sufficient immunity in these populations.

The results of our study show that 2009 H1N1 vaccine is associated with an acceptable safety profile for adults as well as children and elderly people. The level of immunity induced by the first dose of the vaccine appears to be influenced by the presence or absence of alum adjuvant and the age of the recipients (Tables 2 and 3, and Tables 10 through 15 in the Supplementary Appendix). The vaccines formulated without alum adjuvant were more effective in inducing an immune reaction in subjects than were vaccines with adjuvant. This lack of enhancement by the use of alum adjuvant was consistent with data from previous studies of other influenza vaccines.<sup>13,14</sup> There were no significant differences in the immunogenicity of the 15- $\mu$ g and 30- $\mu$ g doses of nonadjuvanted vaccine (Tables 2 and 3), in line with the results reported by Greenberg et al.<sup>12</sup> The immune response to the vaccine varied among the age groups. As in studies of seasonal influenza vaccine,<sup>15-17</sup> age was an important factor associated with the level of induced immunity in our study.

The immune responses in children and the elderly, but not adults or adolescents, can be substantially boosted by a second dose of vaccine (Tables 2 and 3). This finding is consonant with the results of studies evaluating the effectiveness of seasonal influenza vaccination, which showed that one dose of vaccine was highly immunogenic in healthy adults under 65 years of age and that a second dose did not substantially enhance the antibody response.<sup>18-20</sup>

In our study, depending on the age of the subjects, the administration of a single 15- $\mu$ g dose of vaccine is associated with a proportion of subjects with a titer of 1:40 or more of 74.5% (95% confidence interval [CI], 65.1 to 82.5) to 97.1% (95% CI, 91.9 to 99.4) and a geometric mean titer of 64.1 (95% CI, 51.1 to 80.3) to 430.7 (95% CI, 330.0 to 562.1). These results are consistent with the statutory or regulatory requirements of most governments for the use of vaccines.<sup>10,21</sup>

Although one dose of 15  $\mu$ g of vaccine without adjuvant protects the majority of persons, another dose given 21 days later will increase the antibody response in children. The decision about whether to administer two doses of vaccine in children will need to be made by public health officials. One argument for a two-dose vaccine schedule in children is the unusual epidemiology of the 2009 H1N1 pandemic: it affects younger age groups, including young children, and significant morbidity and mortality appear to occur in these younger age groups.<sup>5</sup>

Supported by Hualan Biological Bacterin Company.

Drs. Lin, Xi, and Yang report being employees of Hualan Biological Bacterin Company. No other potential conflict of interest relevant to this article was reported.

We thank the following members of the research and development team from Hualan Biological Bacterin Company for their critical role in this study: Kang An, Bei Fan, Ruowen Pan, Xiaowei Ma, Daoyuan Chen, Yongchao Zhang, Xianpu Yang, Ling Zhu, and Wei Zhang; the following staff members at the School of Public Health, Southeast University, for kindly providing statistical analysis: Prof. Pei-Liu, Jing-Xin Li, Jing-Fang Sun, and Chao-Yun Li; the Excel Pharmastudies Vaccine Center for monitoring the study subjects; and Drs. Zhang Jun and Anthony Yeo from Xiamen University and Drs. Qian Bian and Ye-Fei Zhu from the Jiangsu Center for Disease Control and Prevention for their help in writing the first draft of the manuscript.

---

## APPENDIX

In addition to the authors, the following investigators, from Taizhou Municipal Center for Disease Control and Prevention of China, contributed to the trial: Z.-L. Ma, X.-L. Zhao, Q.-H. Yi, H.-D. Guo, P. Tai, W.-H. Huang, X.-M. Zhang, Z.-K. Zhu, W.-J. Dai, J.-F. Chen, Y.-B. Xu, J. Wang, H. Kan, H.-Y. Cai, P. Zhang, S.-Q. Wang, Y. Zhou, Q. Tang, Q.-Y. Cai, Y.-L. Liu, B. Liu, C.-X. Diao, H.-W. Zhou, Y.-L. Chu, R.-Y. Chen, G.-X. Xu, H. Sheng, X.-L. Jiang, W.-B. Xu, Y.-M. Lv, J.-P. Yang, S.-Q. Cao, D.-M. Xie, K.-X. Yang, Y.-Y. Jiang, Z. Cai, Y. Xu, C. Wang, X.-B. Lu, H.-Y. Yang, J.-L. Zhang, D.-K. Zhang, L. Cao, Y.-C. He, X.-X. Zhu, H. Xiao, J. Shen, W.-M. Huang, X. Zhang, X.-B. Xu, Hua-M. Yang.

## REFERENCES

1. Update: swine influenza A/H1N1 infections — California and Texas, April 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:435-7.
2. Swine influenza A/H1N1 infection in two children — Southern California, March–April 2009. *MMWR Morb Mortal Wkly Rep* 2009;58:400-2.
3. New influenza A/H1N1 virus: global epidemiological situation, June 2009. *Wkly Epidemiol Rec* 2009;84:249-57.
4. Serum cross-reactive antibody response to a novel influenza A (H1N1) virus after vaccination with seasonal influenza vaccine. *MMWR Morb Mortal Wkly Rep* 2009;58:521-4.
5. Use of influenza A (H1N1) 2009 monovalent vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 2009;58(RR-10):1-8.
6. World Health Organization. Availability of a candidate reassortant vaccine virus for the novel Influenza A (H1N1) vaccine development X-179A. (Accessed October 16, 2009, at [http://www.who.int/csr/resources/publications/swineflu/candidates\\_X-179a/en/](http://www.who.int/csr/resources/publications/swineflu/candidates_X-179a/en/))
7. Division of Microbiology and Infectious Diseases. Adult toxicity table. (Accessed October 16, 2009, at <http://www3.niaid.nih.gov/LabsAndResources/resources/DMIDClinRsrch/toxtables.htm>.)
8. *Idem*. Pediatric toxicity table. (Accessed October 16, 2009, at <http://www3.niaid.nih.gov/LabsAndResources/resources/DMIDClinRsrch/toxtables.htm>.)
9. Wu J, Fang HH, Chen JT, et al. Immunogenicity, safety, and cross-reactivity of an inactivated, adjuvanted, prototype pandemic Influenza (H5N1) vaccine: a phase II, double-blind, randomized trial. *Clin Infect Dis* 2009;48:1087-95.
10. European Committee for Proprietary Medicinal Products. Note for guidance on harmonisation of requirements for Influenza vaccines (CPMP/BWP/214/96). London: European Agency for the Evaluation of Medicinal Products, 1997.
11. Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines. Bethesda, MD: Food and Drug Administration, May 2007.
12. Greenberg ME, Lai MH, Hartel GF, et al. Response after one dose of a monovalent influenza A (H1N1) 2009 vaccine — preliminary report. *N Engl J Med* 2009;361. DOI: 10.1056/NEJMoa0907413.
13. Bresson JL, Perronne C, Launay O, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. *Lancet* 2006;367:1657-64.
14. Ehrlich HJ, Müller M, Oh HML, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. *N Engl J Med* 2008;358:2573-84.
15. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of influenza: recommendations for influenza immunization of children, 2007-2008. *Pediatrics* 2008;121(4):e1016-e1031.
16. Gross PA, Weksler ME, Quinnan GV Jr, Douglas RG Jr, Gaerlan PF, Denning CR. Immunization of elderly people with two doses of influenza vaccine. *J Clin Microbiol* 1987;25:1763-5.
17. Levine M, Beattie BL, McLean DM. Comparison of one- and two-dose regimens of influenza vaccine for elderly men. *CMAJ* 1987;137:722-6.
18. Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. *Pediatrics* 2005;115:1039-47.
19. Neuzil KM, Jackson L, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naïve 5-8-year-old children. *J Infect Dis* 2006;194:1032-9.
20. Ritzwoller DP, Bridges CB, Shetterly S, Yamasaki K, Kolczak M, France EK. Effectiveness of the 2003–2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. *Pediatrics* 2005;116:153-9.
21. European Committee for Proprietary Medicinal Products. Guideline on dossier structure and content for pandemic Influenza vaccine marketing authorisation application (CPMP/VEG/4717/03). London: European Agency for the Evaluation of Medicinal Products, 2004.

Copyright © 2009 Massachusetts Medical Society.