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Comparative immunogenicity of recombinant influenza hemagglutinin (rHA) and trivalent inactivated vaccine (TIV) among persons ≥65 years old☆,☆☆

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ABSTRACT

Alternative substrates for influenza vaccine production are needed to ensure adequate supplies. We evaluated the relative safety and immunogenicity of recombinant hemagglutinin (rHA) or trivalent inactivated vaccine (TIV) among 869 ≥65-year-old subjects in a randomized clinical trial. Virologic surveillance for influenza-like illness (ILI) was conducted during the 2006–2007 epidemic. Vaccines were well tolerated. Seroconversion rates vs. influenza A/H1N1 and H3N2 antigens were superior in the rHA group, but were inferior vs. influenza B; however, results for influenza B are confounded since the vaccine antigens were different. ILI frequencies were low and similar in both groups. Studies assessing relative immunogenicity of vaccines using identical B Ags are warranted.

1. Introduction

Annual influenza epidemics are associated with serious excess morbidity and mortality, particularly among the elderly [1–3]. Licensed trivalent inactivated influenza vaccines (TIVs) have been shown to reduce hospitalization and death following influenza in this vulnerable population [4–8], but their efficacy is lower than that observed in younger, healthy populations. In addition, recent studies have questioned the level of effectiveness of TIV in the elderly, suggesting that cohort studies have overestimated the benefits of immunization with current TIV formulations in this age group [9,10]. In view of these considerations, it is widely accepted that improved and alternative vaccines are needed for control of seasonal and pandemic influenza.

Currently available TIVs are prepared from viruses that are grown in embryonated hens' eggs [11]. Alternative substrates for vaccine production are desirable in order to reduce the vulnerability of, and to expand influenza vaccine supply [12]. Recombinant DNA techniques allow for expression of the influenza hemagglutinin (rHA) by baculovirus vectors in insect cell cultures [13]. Advantages of this technique include speed of production, absence of egg protein, and a highly purified product. Previous studies among healthy younger and older adults have confirmed that rHA vaccines are safe, well tolerated and immunogenic at dosages up to nine times higher than those contained in TIV [14–17]. Dose-related increases in serum antibody levels after immunization also were observed.

The goals of the current study were to obtain additional evidence in support of the safety and immunogenicity of an experimental rHA vaccine in an elderly population, and to establish non-inferiority of the immunogenicity of the rHA vaccine when compared with a licensed TIV. A secondary objective was to provide a preliminary estimate of the relative efficacy of the two vaccines against culture-positive influenza-like illness during the subsequent epidemic.

2. Materials and methods

2.1. Study design

We conducted a phase 3 multicenter, randomized, double-blind, controlled clinical trial. Written informed consent was obtained

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from potential subjects prior to screening. Ambulatory, community dwelling immunocompetent persons who were ≥65 years old, who had no known allergy to vaccine components (including eggs), and who were medically stable were considered eligible. The study was conducted in accordance with protocols approved by Institutional Review Boards at the participating study sites.

2.2. Vaccines

The licensed trivalent inactivated influenza (TIV) contained representative strains for the 2006–2007 influenza season: A/Wisconsin (H3N2); A/New Caledonia (H1N1) and B/Malaysia (Fluzone®, sanofi pasteur). Each 0.5 mL dose contained 15 μg of influenza hemagglutinin (HA) of each strain (45 μg total). The composition of the trivalent recombinant hemagglutinin (rHA) vaccine was similar, with the exception that the influenza B antigen was B/Ohio, a related variant. Each 0.5 mL dose of the rHA vaccine contained ~45 μg/strain (135 μg total) of rHA (FluBlok®; Protein Sciences Corporation), as determined in a single radial immunodiffusion assay. The total protein content of the vaccine used in this study was 131 μg as determined by BCA (Bicinchoninic acid or modified Lowry method).

2.3. Procedures

2.3.1. Vaccination

Eligible subjects were stratified according to whether they received influenza vaccine during the 2005–2006 influenza season, and then randomly assigned to receive a single dose of TIV or rHA in the non-dominant deltoid muscle. Vaccinations were administered by personnel who were not involved in the assessment of responses after immunization.

2.3.2. Safety assessments

Subjects were observed for 30 min after each immunization. For 7 days after each immunization, subjects recorded their oral temperature and the presence and severity of injection site (pain, tenderness, redness and swelling) and systemic symptoms (feverishness, malaise, myalgia, headache and nausea) on a memory aid. Subjects were contacted by telephone 8–10 days after immunization, at which time their memory aids were reviewed by study staff. Before and 28 days after immunization and at the end of the 2006–2007 influenza season (EOIS), the medical history was reviewed.

The severity of solicited adverse events (AEs) was scored on a scale from 0 to 3, where 0 = absence of the symptom; 1 = mild symptom that did not interfere with activity; 2 = moderate symptom that interfered with activity; and 3 = severe, incapacitating symptom. Injection site redness and swelling were graded on the diameter of measurement, as follows: 0 = <1 cm; 1 = small (1 to <2 cm); 2 = medium (2 to <5 cm); and 3 = large (≥5 cm). Fever was defined as an oral temperature ≥99.6 °F for this elderly population. Serious adverse events (SAEs) were defined as life-threatening AEs, or AEs that resulted in significant or persistent disability, hospitalization, or death. All reported AEs that occurred during the 28 days after vaccination were recorded, as were all reported SAEs that occurred during the entire study period.

2.3.3. Immunogenicity assessments

Blood samples for antibody assays were collected before and 1 month after immunization, and at the EOIS. Serum hemagglutination-inhibition (HAI) assays were performed by a central laboratory (Cincinnati Children's Hospital Medical Center), as described previously [17] with the following modifications: the initial sample dilution was 1:10; serum samples with no reactivity were assigned a titer of 5; influenza test antigens used in the HAI assay were prepared by Protein Sciences Corporation using the baculovirus expression system (and matched the antigens contained in the rHA vaccine), except for the B/Malaysia antigen used in the "ad hoc" analysis, which was egg-derived and obtained from Centers for Disease Control and Prevention; and turkey red blood cells were used in all assays. A fourfold or greater increase in antibody titer between day 0 and 28, or between day 28 and EOIS was considered significant.

2.3.4. Surveillance for influenza-associated and influenza-like illness (ILI)

Subjects who experienced ILI symptoms completed a weekly "Flu Symptoms Card" to record symptoms of acute illness, and to continue to record symptoms for as long as they persisted. During the influenza surveillance period (defined below), subjects received weekly phone calls to ascertain the presence or absence of symptoms listed on the Flu Symptoms Card. Subjects were also reminded to contact the clinic for illness evaluation if they recorded an influenza symptoms score of 2 or greater on the Flu Symptoms Card (based on presence of fever, cough, sore throat, and runny nose/stuffy nose, muscle or joint aches, headache, chills/sweats, and tiredness/malaise), or if they sought medical care for their acute illness.

Participating sites conducted active surveillance for influenza or had access to reports of positive isolates from local clinical laboratories. Active surveillance for influenza began when two or more positive cases had been detected in community surveillance or laboratory reports. Surveillance ended after 3 consecutive weeks without a positive sample from either community surveillance or from study subjects, unless reports from national (CDC) surveillance showed continued circulation of influenza due to a strain that had not already occurred at that study site. Flu surveillance for the individual subject ended upon completion of the EOIS visit.

2.3.5. Illness assessments

Subjects reporting an ILI score of 2 or greater on their Flu Symptoms Card were asked to come to the clinic for an illness evaluation. At this visit the subject's medical history was reviewed and a notation made of any changes the subject's health status, and a physical examination was performed. Nasal and throat swabs (NS/TS) were obtained if the subject met CDC-ILI definition (fever with cough and/or sore throat) and/or if the subject had sought medical care at another location (medically attended acute respiratory illness, or MAARI); these were placed in viral transport media. Samples were refrigerated at 2–10 °C storage for up to 24 h and then frozen at −70 °C.

2.3.6. Isolation of influenza viruses

(NS/TS) specimens were shipped to a central laboratory (Cincinnati Children's Hospital Medical Center) for processing. Virus isolation was carried out using standard cell culture methods in primary rhesus monkey kidney (PRhMK) cells. Briefly, clinical samples were absorbed onto PRhMK monolayers for approximately 60 min, and then monitored daily for cytopathic effect up to 14 days. If a cytopathic effect score of 2+ was reached, the presence (or absence) of either influenza A or B was determined by fluorescent antibody testing with monoclonal antibodies (Diagnostic Hybrids, Inc.). All clinical samples were tested by fluorescent antibody testing at the end of the 14-day incubation period to confirm.

2.4. Statistical considerations

The primary objective of this study was to compare the immunogenicity of a trivalent rHA vaccine to that of a U.S. licensed TIV among ambulatory adults who were ≥65 years old. Secondary objectives were to compare the safety and reactogenicity

of TIV and the rHA vaccine, and to compare the relative efficacy and effectiveness of the two vaccines for prevention of culture-positive CDC-ILI and/or culture-positive MAARI during the 2006–2007 influenza epidemic season. Primary endpoints were the proportions of subjects in each group who seroconverted after immunization and geometric mean titers (GMTs) of serum antibodies against vaccine antigens 28 days after immunization. Secondary endpoints included frequencies of adverse events (AEs) and serious AEs (SAEs); proportions of subjects achieving a serum HAI titer of ≥40 after immunization; and the proportion of subjects in each vaccine group who experienced culture-positive CDC-ILI and/or culture-positive MAARI during the 2006–2007 influenza season.

The primary study hypothesis was that serum HAI antibody responses following immunization with the rHA vaccine would be non-inferior to those elicited by immunization with TIV using FDA Guidance Document criteria for seasonal influenza vaccine [18]. To ensure sufficient power to test for non-inferiority of rHA vs. TIV requires demonstration of non-inferiority for two co-primary endpoints for each viral strain represented in the vaccine (for a total of six co-primary endpoints). These include [1] GMT and [2] seroconversion rates. FDA Guidance recommendations indicate that [1] the upper bound of the two-sided 95% CI on the ratio of the GMTs should not exceed 1.5; and [2] the upper bound of the two-sided 95% CI on the difference between the seroconversion rates in the two study groups should not exceed 10%. This requires that the α for each constraint be equal to .05 (two tailed). Power, however, must be specified to an overall level. Thus, all six individual comparisons must be constructed at a level of .05 (two tailed), for an overall power of 96.34%. Based on historic seroconversion rates and GMTs for rHA and TIV, a minimum of 655 subjects per arm would be required to ensure 80% power for the test of non-inferiority of rHA to TIV.

3. Results

Between October 9 and December 6, 2006, 870 subjects were enrolled; 869 of these were vaccinated and evaluable for safety evaluations: 436 and 433 in the group given rHA and TIV, respectively. 431 and 430 subjects in the rHA and TIV groups, respectively, provided paired serum samples for antibody assays and were evaluable for immunogenicity endpoints, of whom ~84% were vaccinated in the prior season (359/431 vs. 363/430 in the rHA and TIV groups, respectively; p = NS; Fig. 1). Baseline characteristics and preimmunization serum antibody levels were similar in the two groups (Table 1).

3.1. Safety and reactogenicity

Four subjects died during the course of the study, 2 in each vaccine group. None of the deaths was judged as being related to vaccination. No other SAEs judged to be associated with immunization were reported.

3.2. Solicited adverse events (Fig. 2)

The overall frequencies of subjects who reported any solicited reactogenicity events during the week after immunization were similar in the two vaccine groups (50% of TIV recipients vs. 47% of rHA recipients). Among the rHA group, 37% of subjects reported grade 1 reactions, 8% grade 2, and 2% grade 3 reactions, vs. 40%,

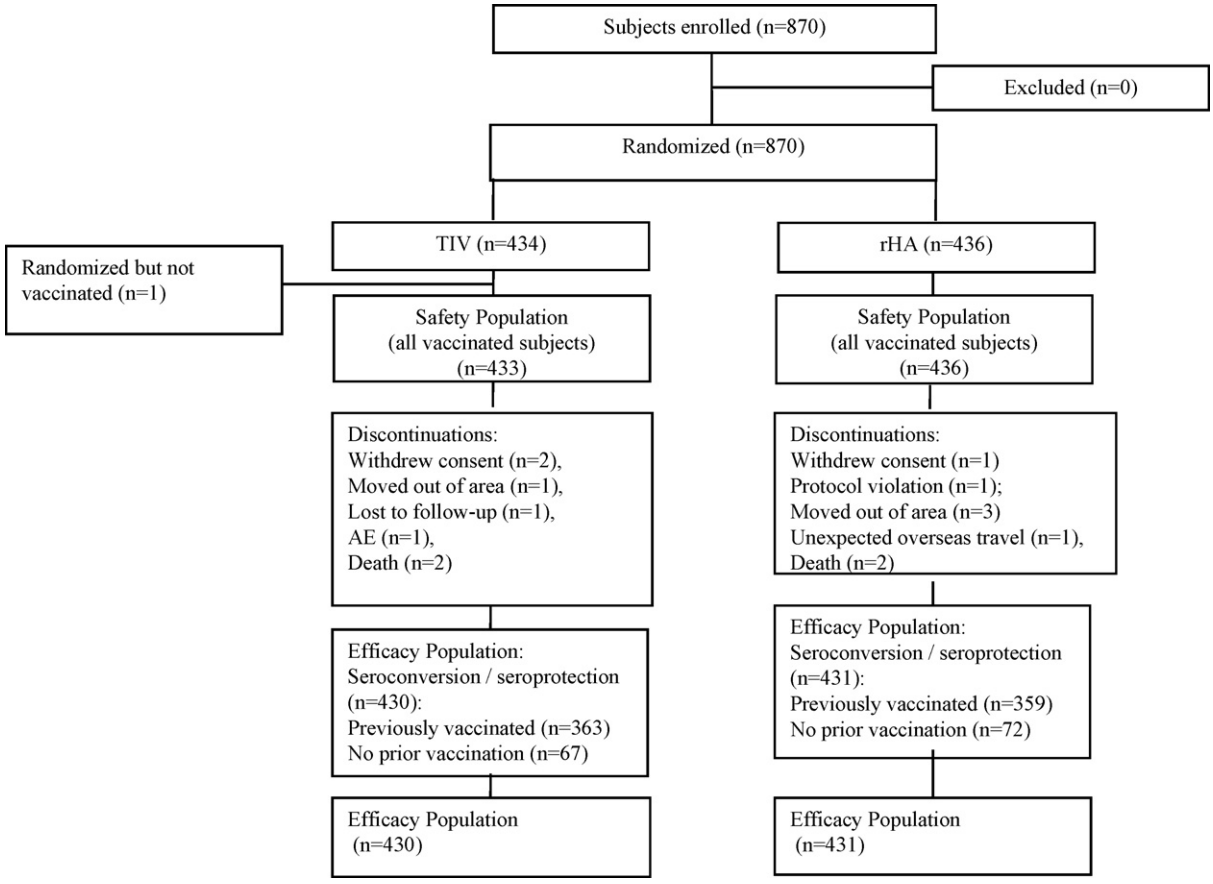

Fig. 1. Disposition of enrolled subjects.

Table 1
Demographic and baseline serologic characteristics of enrolled subjects.

Vaccine	Sex (M:F)	Mean age in years (S.D.)	Vaccinated the previous season (yes/no)[a]	Race/ethnicity[b]	Geometric mean serum HAI antibody levels before immunization (percent with titer <40)[a]		
					H3N2	H1N1	B
rHA (N = 436)	208:228	72.9 (6.66)	359/72	432:2:1:0:1	42.7 (41.8)	69.0 (20.2)	79.9 (20.4)
TIV (N = 433)	199:234	73.0 (6.13)	363/67	420:7:0:2:4	44.7 (38.8)	70.2 (24.4)	80.3 (18.8)
p-value	NS	NS	NS	NS	NS	NS	NS

[a] Evaluable population.
[b] Number of subjects who were White:Black:Hispanic:Asian:other.

7%, and 3% of subjects, respectively, of TIV recipients (p = 0.52; Cochran–Mantel–Haenszel chi-square test).

3.3. Injection site reactogenicity

Of the five injection site reactogenicity events that were reported on the memory aid during the week after immunization, injection site discomfort was the most commonly reported (22% in the rHA group vs. 23% in the TIV group; p = NS). The frequencies of injection site redness (10% and 12%) and swelling (7% and 10%) reported among subjects given rHA or TIV, respectively, also were low and similar in the two groups. Most injection-site reactions were mild and transient.

3.4. Systemic reactogenicity

Of the nine systemic reactogenicity events that were reported on the memory aid during the week after immunization, tiredness/lack of energy was the most commonly reported (14% of rHA recipients vs. 15% of TIV recipients). Headache (11% and 10%) and fatigue (9% and 10%) were the next most common symptoms reported by subjects in the rHA and TIV groups, respectively. Fever during the week after immunization was recorded by fewer than 3% of subjects in each group [N = 9/433 (2.1%) and 11/436 (2.5%) for the TIV and rHA groups, respectively]; and oral temperature of 100 °F or greater was recorded by 5 (1.2%) and 7 (1.6%) subjects in the TIV and rHA groups, respectively. In general, the frequency and severity of the other systemic events were similar in the two study groups.

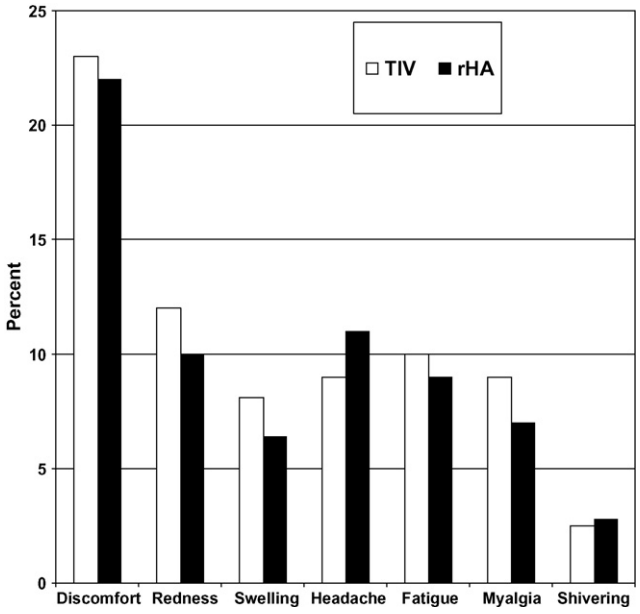

Fig. 2. Percent of subjects reporting solicited injection site and systemic reactions during the week after immunization. TIV = trivalent inactivated influenza virus vaccine; rHA = trivalent recombinant hemagglutinin vaccine.

The proportions of subjects in each group who experienced at least one treatment-emergent or unsolicited AE after immunization were similar (21% in the rHA group vs. 20% of the TIV group; data not shown). In general, most reactions were clinically mild and self-limited. Ten subjects in the rHA group were noted by study staff to have injection site erythema immediately following vaccination vs. 1 subject in the TIV group (p < 0.01; z-test for two proportions). However, most of these reactions resolved within several days and were not accompanied by other injection-site reactions such as pain or induration (see Section 3.3).

3.5. Immunogenicity

Serum HAI antibody responses according to age are summarized in Table 2. Seroconversion rates among rHA recipients satisfied the CBER-specified non-inferiority criterion with respect to differences in seroconversion rates for both influenza A antigens: 43% vs. 33% of subjects given rHA or TIV, respectively, developed serum antibody responses vs. A/New Caledonia (H1N1) (p = 0.001); and 78% vs. 58%, respectively, developed significant increases in serum HAI antibody vs. A/Wisconsin (H3N2) (p < 0.001). Moreover, the lower bounds of the two-sided 95% CI of the seroconversion rates in the rHA group exceeded the criterion of ≥30% (the "placebo controlled" criterion specified by CBER for elderly subjects), whereas in the TIV group, this criterion was met for H3 but not H1 (Table 3). These differences were even more pronounced in an exploratory analysis that examined antibody responses in subjects who were ≥75 years old.

In contrast to the results for influenza A antigens, the CBER-specified non-inferiority criterion for the difference in seroconversion rates for all subjects vs. influenza B/Ohio was not achieved for rHA. The seroconversion rate in the rHA group was 29%, as compared with 39% in the TIV group (upper bound of the two-sided 95% CI of the difference in seroconversion rates = 16.1%). However, these findings are confounded by the lack of a head-to-head comparison for the influenza B antigen. The seroprotection rate in rHA recipients (i.e., proportion of subjects with a post-vaccination titer ≥40) was 92% (two-sided 95% CI: 88.6–94.1). Thus, the seroprotection rate for rHA group for the influenza B antigen satisfies the "placebo controlled" criterion in the Guidance Document; i.e., that the lower bound of the two-sided 95% CI for the percent of subjects achieving an HI antibody titer ≥40 should meet or exceed 60%.

The ratio of the GMT for TIV to the GMT for rHA at day 28 was a pre-specified co-primary endpoint in the study. The upper bounds of the two-sided 95% CI for the GMT ratios for the H1, H3 and B antigens were 0.86, 0.60, and 1.34, respectively. Thus, the criterion for non-inferiority was met for all three strains (Table 3). These criteria were also met in the subpopulation of subjects aged 75 years and older.

Serum antibody responses according to receipt of TIV in the previous season (yes/no) are detailed in Table 4. As expected,

Table 2
Serum hemagglutination inhibition antibody responses following immunization with rHA or TIV according to age group.

Response parameter	Age group			
	≥65 years old		≥75 years old	
	rHA (*N* = 431)	TIV (*N* = 430)	rHA (*N* = 163)	TIV (*N* = 159)
Influenza A/New Caledonia (H1N1)				
GMT pre	69.0 (62.1, 76.6)	70.2 (62.8, 78.6)	63.3 (53.6, 74.8)	65.5 (55.3, 77.6)
GMT post	176.8 (159.4, 196.0)	148.1 (134.2, 163.4)	152.7 (128.1, 182.0)	125.3 (107.1, 146.7)
GMT ratio; TIV:rHA	0.84 (0.81, 0.86)		0.82 (0.79, 0.85)	
% ≥40 post	95 (92, 97)	95 (92, 97)	91 (87, 96)	94 (91, 98)
% with rise	43 (39, 48)	33 (28, 37)	39 (32, 47)	30 (23, 37)
Influenza A/Wisconsin (H3N2)				
GMT pre	42.7 (37.6, 48.4)	44.7 (39.2, 51.0)	39.7 (32.7, 48.1)	43.1 (35.2, 52.8)
GMT post	338.5 (299.7, 382.5)	199.2 (176.8, 224.4)	300.2 (244.7, 368.3)	178.4 (147.8, 215.3)
GMT ratio; TIV:rHA	0.59 (0.57, 0.60)		0.59 (0.58, 0.61)	
% ≥40 post	97 (94, 98)	93 (90, 95)	96 (93, 99)	93 (89, 97)
% with rise	78 (74, 82)	58 (53, 62)	79 (73, 85)	54 (46, 62)
Influenza B/Ohio				
GMT pre	79.9 (71.3, 89.5)	80.3 (72.0, 89.5)	101.9 (86.7, 119.9)	102.6 (86.1, 122.1)
GMT post	149.6 (134.5, 166.3)	194.8 (177.5, 213.7)	185.7 (160.8, 214.4)	224.8 (193.2, 261.5)
GMT ratio; TIV:rHA	1.30 (1.26, 1.34)		1.21 (1.18, 1.24)	
% ≥40 post	92 (89, 94)	97 (95, 99)	96 (93, 99)	99 (98, 100)
% with rise	29 (25, 34)	39 (34, 44)	26 (22, 33)	35 (28, 43)
Influenza B/Malaysia (post hoc analysis)				
GMT pre	12.1 (11.1, 13.3)	12.6 (11.5, 13.8)	14.4 (12.3, 16.8)	14.9 (12.9, 17.2)
GMT post	16.5 (15.0, 18.3)	22.6 (20.4, 25.2)	18.1 (15.6, 21.1)	25.3 (21.1, 30.3)
GMT ratio; TIV:rHA	1.37 (1.0, 1.7)		1.40 (1.1, 1.7)	
% ≥40 post	30 (25.8, 34.5)	40 (35.4, 44.6)	31 (24.2, 38.4)	47 (38.8, 54.3)
% with rise	10 (7.1, 12.8)	20 (16.0, 23.5)	6 (2.5, 9.8)	19 (13.3, 25.7)

Table 3
CBER criteria for non-inferiority of rHA vs. TIV.

Parameter	CBER criterion	Influenza A/H3N2	Influenza A/H1N1	Influenza B/Ohio
Seroconversion rates	Upper bound of the 95% CI on the difference between seroconversion rates (licensed vaccine–new vaccine) ≤ 10%	−20.1% (−26.2, −13.9); $p < 0.001$	−10.8% (−17.3, −4.4); $p = 0.001$	9.8% (3.5, 16.1); $p = 0.002$
	Satisfies criterion?	Yes	Yes	No
GMT ratio	Upper bound of the two-sided 95% CI on the ratio of the GMTs (GMT licensed vaccine/GMT new vaccine) ≤ 1.5	0.59 (0.57, 0.60)	0.84 (0.81, 0.86)	1.30 (1.26, 1.34)
	Satisfies criterion?	Yes	Yes	Yes

Table 4
Serum HAI antibody responses according to prior vaccination status.

Response parameter	Immunization group			
	No vaccine in the previous season		Vaccinated during the previous season	
	rHA (*N* = 72)	TIV (*N* = 67)	rHA (*N* = 359)	TIV (*N* = 363)
Influenza A/New Caledonia (H1N1)				
GMT pre	41.6 (31.1, 55.5)	47.2 (33.7, 66.12)	76.4 (68.5, 85.2)	77.6 (67.3, 84.9)
GMT post	246.8 (190.0, 320.4)	225.1 (168.8, 300.2)	165.3 (147.9, 184.8)	137.1 (123.8, 151.7)
GMT ratio; TIV:rHA	0.91 (0.89, 0.93)		0.83 (0.8, 0.86)	
% ≥40 post	97 (93.4, 100)	97 (92.9, 100)	94 (91.7, 96.6)	94 (92.1, 96.8)
% with rise	75 (65.0, 85.0)	55 (43.3, 67.1)	37 (32.1, 42.0)	28 (23.7, 33.0)
Influenza A/Wisconsin (H3N2)				
GMT pre	23.8 (17.9, 31.6)	21.1 15.8, 28.2)	47.96 (41.79, 55.04)	51.37 (44.55, 59.22)
GMT post	419 305.7, 574.2)	257.5 (179.3, 369.9)	324.4 (284.3, 370.1)	190.0 (167.9, 215.1)
GMT ratio; TIV:rHA	0.61 (0.6, 0.63)		0.59 (0.57, 0.60)	
% ≥40 post	96 (91.2, 100)	91 (84.2, 97.9)	97 (94.8, 98.5)	93 (90.2, 95.5)
% with rise	92 (85.3, 98.1)	84 (74.7, 92.5)	75 (70.4, 79.4)	53 (47.8, 58.0)
Influenza B/Ohio				
GMT pre	56.57 (42.2, 75.84)	68.50 (49.73, 94.35)	85.6 (75.77, 96.69)	82.64 (73.68, 92.69)
GMT post	137.16 (100.35, 187.47)	252.24 (195.9, 324.79)	152.17 (136.2, 170.01)	185.70 (168.22, 204.98)
GMT ratio; TIV:rHA	1.84 (1.77, 1.91)		1.22 (1.19, 1.25)	
% ≥40 post	85 (76.4, 93.0)	97 (92.9, 100)	93 (90.4, 95.7)	97 (95.6, 98.9)
% with rise	43 (31.6, 54.5)	52 (40.3, 64.2)	26 (21.9, 31.0)	37 (31.7, 41.6)

serum antibody titers before immunization were higher in subjects who reported receiving TIV during the previous season, and the proportions of subjects with significant responses following immunization were higher in the groups that were not previously vaccinated.

3.6. Efficacy

A secondary endpoint in the study was the relative efficacy of rHA (in comparison to TIV) in the prevention of culture-confirmed CDC-ILI or MAARI. Of 433 subjects given TIV, 28 (6.5%) had nasal wash/throat swab specimens collected for isolation of influenza virus vs. 25/436 (5.8%) of subjects given rHA (p = NS). No significant differences in the rates or culture-confirmed ILI or any ILI were observed between the two vaccine groups. Only one case (0.2%) of culture-confirmed CDC-ILI occurred in the rHA group vs. two (0.5%) in the TIV group (p = NS). Eight additional subjects in the rHA group had serologic evidence of influenza infection (1 H1N1, 1 H3N2, and 6 influenza B titer rises); while 11 subjects (one with a positive culture) had a significant rise in antibody titer between the day 28 specimen and the EOIS in the TIV group (3 H1N1, 2 H3N2 and 6 influenza B titer rises). Therefore, 9/436 (2%) of rHA subjects vs. 12/433 (3%) of TIV subjects had laboratory-confirmed influenza (p = NS). There were also very few cases of CDC-ILI, regardless of culture results: 27 subjects (6.2%) in the rHA group and 28 cases (6.5%) in the TIV group (p = NS).

4. Discussion

Our results confirm and extend those reported previously. Both TIV and rHA were safe and well tolerated among ambulatory elderly subjects. The most common vaccine-associated adverse event during the week after immunization was transient injection site discomfort. The frequency and severity of injection site and systemic reactions after immunization were similar in both groups. The co-primary endpoint for non-inferiority of GMTs was met for all 3 vaccine antigens among subjects given rHA when compared to subjects given TIV (i.e., none of the upper bounds of the two-sided 95% CI on the ratios of the GMTs exceeded 1.5). Notably, the GMTs of serum HAI antibody vs. influenza A/H1N1 and A/H3N2 antigens were significantly higher in the group given rHA. Seroconversion rates (the second co-primary endpoint) vs. influenza A/H1N1 and A/H3N2 vaccine antigens also were significantly higher among subjects given rHA than those given TIV.

In contrast to antibody responses against influenza A viruses, the GMT of serum HAI antibody vs. B/Ohio, the antigen contained in the rHA formulation, was significantly higher in the group given TIV. Likewise, the seroconversion non-inferiority endpoint for the B/Ohio antigen was not met for subjects who received rHA containing B/Ohio antigen when compared with those given TIV containing B/Malaysia antigen (i.e., the upper bound of the two-sided 95% CI on the difference between the seroconversion rates between rHA and TIV was 16.4% instead of <10%). The antigens present in the 2 vaccine formulations were related but not identical; therefore, the significance of this difference is not known. Nevertheless, over 90% of subjects in both vaccine groups achieved a titer of 40 or greater after immunization. Further studies assessing the relative immunogenicity of the 2 vaccines using identical influenza B antigens are warranted.

Of particular importance was the observation that the patterns of immune responses against both influenza A antigens were similar among the group as a whole and subjects who were 75 years of age or older, a subpopulation that is at the greatest risk of severe influenza and death following influenza [19]. In view of the fact that influenza A/H3N2 viruses have been responsible for the majority of excess hospitalizations and deaths attributable to influenza over the past four decades, our observations of superior immunogenicity of the rHA vaccine are particularly relevant. Serum HAI antibody titers of 40 or greater after immunization has been used as a predictor of likely protection against naturally acquired influenza; however, virologic confirmation of infection provides a direct measure of vaccine efficacy. A secondary endpoint of the study was to assess relative efficacy of the two vaccines against influenza during the subsequent epidemic. The rates of culture-positive ILI were low and similar in both groups. Therefore, definitive conclusions regarding relative efficacies cannot be drawn from this trial. Expanded efficacy studies with larger sample sizes would be necessary in order to draw definitive conclusions regarding relative efficacies of the licensed and rHA vaccines.

The potential of the insect cell–baculovirus technology to facilitate expeditious responses to health care emergencies such as the one currently posed by the H1N1 pandemic is demonstrated by the fact that the first batches of a rH5 vaccine that was used for testing in human subjects was a recombinant influenza A/Hong Kong/97 HA. More recently, a candidate vaccine was available 6 weeks after initiation of the development of a rHA vaccine against the H1N1 A/California/04/2009. Furthermore, this manufacturing technology can readily be transferred to other countries, which would enable rapid expansion and availability of this vaccine. For example, in Korea a 50,000-L capacity exists that could supply millions of vaccine doses in a relatively short time. Such technology transfer would also avoid the serious political impediments to exporting vaccine from manufacturing countries during a pandemic, as occurred when the U.S. closed its borders in 1976 for vaccine export. Shipment of a recombinant baculovirus that can be used to produce vaccine would generally not be limited such regulations. Finally, the cost of producing rHA vaccine using the insect cell–baculovirus system is approximately equivalent to that of the trivalent inactivated influenza vaccine.

The current pandemic has once again highlighted the need for alternate strategies for influenza vaccine production, as several manufacturers have indicated that novel H1N1 virus yields in eggs are significantly lower than for seasonal strains of influenza. The clinical trial data we report provide additional evidence in support of further development of this promising vaccine production strategy.

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