Vaccine 29 (2011) 7733–7739

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (FluBlok®) against influenza in healthy adults: A randomized, placebo-controlled trial☆

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ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 27 July 2011
Accepted 27 July 2011
Available online 9 August 2011

Keywords:
Influenza vaccine
Baculovirus expression
Recombinant protein
Clinical trials

ABSTRACT

Background: Development of influenza vaccines that do not use embryonated eggs as the substrate for vaccine production is a high priority. We conducted this study to determine the protective efficacy a recombinant, baculovirus-expressed seasonal trivalent influenza virus hemagglutinin (rHA0) vaccine (FluBlok®).
Methods: Healthy adult subjects at 24 centers across the US were randomly assigned to receive a single injection of saline placebo (2304 subjects), or trivalent FluBlok containing 45 mcg of each rHA0 component (2344 subjects). Serum samples for assessment of immune responses by hemagglutination-inhibition (HAI) were taken from a subset of subjects before and 28 days after immunization. Subjects were followed during the 2007–2008 influenza season and combined nasal and throat swabs for virus isolation were obtained from subjects reporting influenza-like illness.
Results: Rates of local and systemic side effects were low, and the rates of systemic side effects were similar in the vaccine and placebo groups. HAI antibody responses were seen in 78%, 81%, and 52% of FluBlok recipients to the H1, H3, and B components, respectively. FluBlok was 44.6% (95% CI, 18.8%, 62.6%) effective in preventing culture-confirmed influenza meeting the CDC influenza-like illness case definition despite significant antigenic mismatch between the vaccine antigens and circulating viruses.
Conclusions: Trivalent rHA0 vaccine was safe, immunogenic and effective in the prevention of culture confirmed influenza illness, including protection against drift variants.

1. Introduction

Although embryonated hen's eggs have been used to generate effective influenza vaccines for many years, this system does have several important drawbacks. Vaccine manufacturing using eggs requires specialized facilities, and the ability to scale up egg production rapidly in response to an emergency is limited. In addition, poultry are potentially vulnerable to the same subtypes of influenza viruses that might also be responsible for pandemic influenza. It is usually necessary to adapt candidate vaccine viruses for high yield growth in eggs, a process that can be time consuming, is not always successful, and which can select receptor variants that may not be optimally representative of circulating influenza strains [1,2].

Expression of proteins in insect cells using recombinant baculovirus has emerged as a promising technology for vaccine production. New recombinant baculoviruses can be generated quickly from sequence data, protein expression is very efficient under the control of the baculovirus polyhedrin promoter, and post translational modifications of the protein are generally similar to other eukaryotic systems. In previous studies, we have evaluated baculovirus-expressed recombinant influenza virus hemagglutinins (rHA0s) as influenza vaccines in humans. Monovalent and bivalent rHA0s have been well tolerated and immunogenic in

☆ ClinicalTrials.gov Identifier: NCT00539981.

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doi:10.1016/j.vaccine.2011.07.128

young adults and adults 65 and older [3–5]. More recent studies have evaluated rHA0 vaccine formulations in healthy adults, subjects aged 50–64 years of age [6] and subjects 65 and older [7,8]. These studies have consistently shown excellent tolerability and antibody responses similar to those seen with egg-derived influenza vaccines. Finally, in a preliminary field efficacy evaluation, recipients of the rHA0 vaccine had reduced rates of culture-positive CDC-defined influenza-like illness compared with placebo recipients, although the study was small [9].

In the present study, we performed a much larger assessment of the immunogenicity, safety, and protective efficacy of the final formulation of trivalent rHA0 vaccine in a population of healthy young adults.

2. Methods

2.1. Vaccine

The vaccine (FluBlok) used in this study consisted of purified hemagglutinin (HA) proteins produced in insect cells using a baculovirus expression system as previously described [9]. The recombinant HA protein is not cleaved in insect cells and is referred to as rHA0. The trivalent vaccine contained 45 mcg (as measured by the single radial immunodiffusion assay) of each purified rHA0 derived from the A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 influenza viruses recommended for the 2007–2008 influenza season formulated with 0.005% Tween®-20 in 10 mM sodium phosphate buffer pH 7.0 ± 0.4 without a preservative. Genes were cloned into baculovirus using RT-PCR from the same CDC-derived vaccine seed viruses used for the production of licensed inactivated influenza vaccine for that year. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified monovalent materials indicated that hemagglutinin constitutes at least 90% of the total protein. Placebo consisted of normal saline for injection, USP.

2.2. Clinical study design

The study was conducted at 24 centers located across the United States (Appendix) during the 2007–2008 influenza season. Subjects were healthy adults aged 18–49 years inclusive, who did not belong to high priority target groups for influenza vaccination as defined by the Advisory Committee on Immunization Practice [10]. Women of child-bearing potential had a negative urine pregnancy test at the time of randomization and were instructed to use contraception during the course of the study. After screening medical history and physical exam to determine eligibility, subjects were randomly assigned to receive either a single dose of FluBlok, or saline placebo using a permuted block randomization scheme stratified for each study site. Vaccine was administered as a single intramuscular injection in the upper deltoid.

Subjects measured their oral temperature daily and maintained a memory aid for 7 days after vaccination on which they recorded local and systemic reactions graded as “mild” (noticeable but not interfering with normal activities), “moderate” (some interference with normal activities), and “severe” (symptom prevented normal daily activities). Subjects received a phone call at day 7 for review of the memory aid, concomitant medications and medical history.

In addition, subjects from five sites (total, $N = 870$) participated in a serological substudy. At these sites, subjects had serum obtained prior to vaccination, and returned approximately 28 days after vaccination for assessment of serum antibody to influenza virus. All other subjects received a phone call from their medical center for review of interim safety data at day 28. A final phone call occurred at the end of the influenza season.

2.3. Surveillance for influenza

During the influenza season, subjects completed a weekly diary to record influenza symptoms, and after influenza was recognized in the community, subjects received weekly phone calls to review the diary and ascertain the presence or absence of respiratory illness symptoms. Subjects were instructed to return to the clinic for illness evaluations if they observed any acute respiratory symptoms or fever. During these illness visits, symptoms were reviewed, a brief physical exam was conducted, and combined nasal and throat swabs for virus culture were obtained.

2.4. Laboratory assays

Sera from the immunogenicity subset were assessed for antibody to each of the three components of the vaccine by hemagglutination-inhibition (HAI) at the Cincinnati Children’s Hospital using a validated assay based on standard methods. All available sera from vaccine recipients, and a randomly selected subset of approximately 33% of placebo specimens were selected for testing. The antigens used in the assays were baculovirus derived rHAs representing the A/Solomon Islands/03/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 viruses, provided by Protein Sciences Corporation. Sera were treated with neuraminidase (RDE, Denka-Seiken, Japan) to remove non-specific inhibitors of hemagglutination prior to testing, and were tested in serial two-fold dilutions at an initial dilution of 1:10. Sera with no reactivity at 1:10 were assigned a value of 1:5. Assays were performed using turkey red blood cells (Viromed Laboratories, Minnetonka, MN).

Swabs for virus culture were stored at $-70\,^{\circ}C$ and shipped on dry ice to a central laboratory (Cincinnati Children’s Hospital Medical Center, Cincinnati, OH) where virus isolation was performed in primary rhesus monkey kidney (RhMK) cells (Diagnostic Hybrids Inc (DHI), Athens, Ohio). The presence of influenza A or B viruses in the culture was determined by immunofluorescence using type-specific monoclonal antibodies (DHI). All influenza isolates were subsequently subtyped and antigenically characterized based on reactivity to ferret antiserum raised against WHO reference strains.

2.5. Statistical analyses

The primary safety endpoints for this study were the rates and severity of solicited and unsolicited adverse events. The primary immunogenicity endpoints were the rates of 4-fold or greater increases in serum HAI antibody to each of the three vaccine strains comparing pre- and 28-day post-vaccination samples. The primary efficacy endpoint was culture-documented influenza illness, defined as development of a CDC-defined influenza-like illness (CDC-ILI) associated with recovery of influenza virus from a nasopharyngeal swab. CDC-ILI was defined as the presence of documented fever ≥ 100 F plus either sore throat or cough.

Safety of the rHA0 vaccine was evaluated via the frequencies and percentages of subjects experiencing adverse events, and a chi-squared test at a nominal 0.05 level was performed to find any differences in incidence rates between groups. Differences between groups in the proportions of subjects with at least a four-fold increase in HAI antibody were also tested using a Chi-Square test. Protective efficacy (PE) was calculated as (1-RR), where RR is the relative risk of having an event compared to the placebo group. Upper and lower confidence limits for the RR were calculated using the exact conditional binomial procedure.

Table 1
Rates of local and systemic events reported by subjects in the 7 days following vaccination.

Symptoms	No. (%) of subjects with complaints by level of severity in each group					
	FluBlok (N = 2344)			Placebo (N = 2304)		
	Mild	Moderate	Severe	Mild	Moderate	Severe
Any	969 (43)*	195 (9)*	34 (1)	567 (25)	130 (6)	30 (1)
Fever (≥100.4)	8 (<1)	5 (<1)	4 (<1)	5 (<1)	6 (<1)	1 (<1)
Fatigue or lack of energy	250 (11)	78 (3)	12 (<1)	256 (11)	66 (3)	11 (<1)
Shivering or chills	52 (2)	12 (<1)	6 (<1)	54 (2)	13 (<1)	4 (<1)
Joint pain	69 (3)	14 (<1)	6 (<1)	67 (3)	12 (<1)	4 (<1)
Muscle pain	197 (9)*	36 (2)*	6 (<1)	124 (6)	22 (<1)	8 (<1)
Headache	264 (12)	70 (3)	15 (<1)	273 (12)	68 (3)	13 (<1)
Nausea	94 (4)	29 (1)	6 (<1)	74 (3)	25 (1)	10 (<1)
Pain	797 (35)*	52 (2)*	2 (<1)	177 (8)	3 (<1)	1 (<1)
Bruising	68 (3)	6 (<1)	1 (<1)	57 (3)	1 (<1)	1 (<1)

The highest level of severity reported for each subject is shown. Severity was graded as 1 = mild, no interference with daily activities, 2 = moderate, some limitation of activity, and 3 = severe, prevents normal daily activity.

* P < .05 compared to placebo.

The sample size for this study was chosen based on the assumption that FluBlok would be at least 70% efficacious in prevention of symptomatic, culture-proven influenza, and that the placebo attack rate would be 3% or greater. The sample size was then chosen to have 80% power to determine that the lower bound of the 95% confidence interval for protective efficacy was greater than 40%, assuming a 5% attrition rate.

2.6. Informed consent

Written informed consent was obtained from all subjects in the study, and the study was approved by the institutional review boards at each study site prior to initiation of the study and enrollment of subjects.

3. Results

A total of 4648 eligible subjects were randomized 1:1 to receive either FluBlok or Placebo, and 4071 (87.5%) completed all study procedures. The disposition of the study subjects is shown in Fig. 1. Of the 4648 enrolled subjects, 2344 were randomized to receive FluBlok and 2304 were randomized to placebo. The majority of subjects were white (66%) and female (59%). The mean age was 32.5 years. Three subjects were over the enrollment criteria of 49 years or less, so that the age range enrolled was 18–55 years; these three subjects are included in both the safety and efficacy analyses. There were no differences with respect to age, sex or race between the groups.

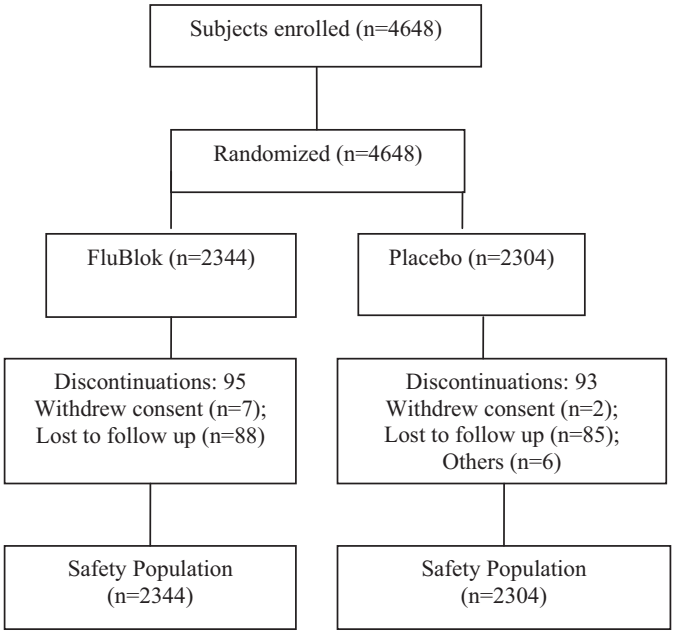

Fig. 1. Flow chart of subject participation in the study.

There were 577 subjects who did not complete the study, the majority of whom were lost to follow-up (260 (11%) in the FluBlok and 251 (11%) in the placebo group). In addition, 36 subjects withdrew consent (22 (1%) and 14 (0.6%) in FluBlok and placebo, respectively), 3 in each group withdrew due to an adverse event (AE), there was 1 death in each group, and 9 and 13 subjects in the FluBlok and placebo groups, respectively were discontinued for other reasons.

3.1. Assessment of vaccine safety

The rates and severities of local and systemic symptoms reported by subjects in the 7 days following administration of FluBlok or placebo are shown in Table 1. FluBlok was associated with local injection site pain and muscle aches that were significantly more frequent than after saline placebo (P < .03 for pain). However, 94% of all complaints of pain after FluBlok were rated as mild. Systemic symptoms following vaccination did not occur at significantly different rates in vaccine and placebo recipients (P > .05 for all comparisons). The most frequently reported systemic symptoms following vaccination were headache (15% in both groups) and fatigue or lack of energy (14.5% in both groups). The majority (76%) of complaints of headache were also mild. There were 17 reports of fever (oral temperature >100.4 F) among FluBlok recipients and 12 among placebo recipients following vaccination.

There were 85 severe adverse events (SAEs) reported during the study in 64 subjects. Of these, 41 SAEs were reported in 30 subjects (1.28%) in the FluBlok group, and 44 SAEs in 34 subjects (1.48%) in the placebo group. Two subjects died during the study; one in each group. A subject in the FluBlok-treatment group experienced a fatal pulmonary embolism approximately three months post vaccination, and a subject in the placebo group had a fatal motor vehicle accident. Both deaths were considered to be unrelated to the study by the site investigator. All but two SAEs (a liposarcoma in a FluBlok recipient and breast cancer in a placebo recipient) had resolved by the end of the study period. None of the 85 SAEs in the study were considered to be definitely or probably related to vaccine. One event, a pericardial effusion in a FluBlok recipient was judged to be possibly related to vaccine. This event occurred in a 47 year-old male with a prior history of hypertension, who began experiencing

Table 2
Serum hemagglutination-inhibiting (HAI) antibody titers in all subjects, and in FluBlok recipients with or without self-reported history of prior seasonal influenza vaccination.

Group	N	Pre (day 0) and post vaccination (day 28) geometric mean antibody titer (95% CI) and response rates (95% CI) to the following vaccine antigens								
		A/Solomon Island/3/2006 (H1N1)			A/Wisconsin/67/2005 (H3N2)			B/Malaysia/2506/2004		
		Pre	Post	%Response	Pre	Post	%Response	Pre	Post	%Response
Placebo	127	20.33 (16.5, 25.0)	18.8 (15.2, 23.3)	3 (1, 8)	21.5 (18.1, 25.4)	15.1 (13.0, 17.6)	3 (1, 8)	31.1 (24.6, 39.4)	19.8 (15.8, 24.8)	0 (0, 3.0)
FluBlok	448	31.9 (28.2, 36.1)	349.0 (316.9, 384.3)	78 (74, 82)	22.9 (20.7, 25.4)	262.1 (234.9, 292.4)	81 (77, 85)	55.27 (49.3, 62.0)	200.55 (181.0, 222.2)	52 (47, 57)
FluBlok PV[a]	93	64.9 (51.0, 82.7)	235.7 (196.1, 283.4)	56 (46, 66)	47.5 (38.6, 58.5)	185.7 (149.4, 230.9)	57 (47, 67)	104.6 (82.0, 133.5)	162.4 (132.4,199.2)	22 (13,30)
FluBlok NPV[b]	355	26.5 (23.1, 30.4)	386.7 (346.7, 431.4)	83 (80, 87)	18.9 (17.0, 21.1)	286.9 (253.3, 324.8)	87 (84, 91)	46.76 (41.3, 52.9)	212.0 (188.6, 238.2)	60 (55, 65)

#Response = 4-fold or greater increase to 1:40 or greater.
[a] PV = previously vaccinated.
[b] NPV = not previously vaccinated.

fever and cough 7 days after vaccination and was hospitalized 11 days after vaccination with a diagnosis of pericardial effusion and cardiac tamponade. Pericardiocentesis showed one colony of *Propionibacterium* spp.; and culture of pleural fluid grew *Staphylococcus epidermidis* only. Viral cultures and a panel of serum antibody tests for viruses causing pericarditis were negative. The subject was discharged home on Day 24 following vaccination and the event was considered resolved by the end of the study period.

There were a total of 37 pregnancies reported during the study; 20 in the FluBlok group and 17 in the placebo group. Complete follow-up information was available for 30 for them (15 in FluBlok and 15 in placebo). Ten of the pregnancies among FluBlok and 8 among placebo recipients were uneventful and resulted in the birth of a normal infant at 36 weeks gestation or later. Two women in the FluBlok and three in the placebo group had associated AEs during their pregnancy which all resolved. Elective or spontaneous abortion occurred in three and four subjects in the FluBlok and placebo groups, respectively.

3.2. Immunogenicity

Serum HAI antibody titers before and after immunization in FluBlok and placebo recipients are shown in Table 2. FluBlok induced serum antibody responses to all three components of the vaccine in the majority of recipients, although lower response rates were seen for the influenza B component. As expected, post vaccination geometric mean titers were substantially higher for all three components in FluBlok than in placebo recipients ($P < .001$ for all comparisons). The proportion of FluBlok recipients with a post-vaccination HAI titer of 1:40 or greater was 99% for the H1 component (95% CI, 97.1%, 99.5%), 97% for the H3 component (94.8%, 98.3%), and 96% for the B component (94.0%, 97.8%).

Among FluBlok recipients, subjects with a self-reported history of seasonal influenza vaccine in the year prior to the study had higher baseline titers of antibody against all three components, and had significantly lower frequencies of HAI response than did those who reported that they had not received vaccine in the previous year. Subjects who reported prior vaccine receipt also had lower post vaccination GMTs for all three components than did those without this history, and this difference was statistically significant for both influenza A components.

3.3. Protective efficacy

Subjects in this study were followed throughout the subsequent influenza season (mid December 2007 to end April 2008) with weekly phone calls and instructed to return to the study clinics for any acute respiratory illness, at which time a combined nasal and throat swab for viral culture was obtained. The primary efficacy endpoint for this study was the development of culture-confirmed CDC-ILI, and we also assessed the effect of vaccination on rates of any influenza culture positive illness.

A total of 582 subjects were cultured for respiratory illness, and influenza virus was isolated from 178 subjects. There were a total of 120 subjects from whom influenza A was isolated, the majority of whom (82/120, 68%) met the CDC-ILI case definition. There were a total of 59 subjects with positive cultures for influenza B, and the majority of these subjects (41/59, 69%) also met the CDC-ILI case definition.

Only 8 isolates in the study (<5% of the total) were antigenically identical to the strains contained in the vaccine. All of these viruses were A/Wisconsin/67/2005-like H3N2 viruses. Two of these occurred in FluBlok recipients and 6 occurred in placebo recipients, and among these subjects one FluBlok recipient and 5 placebo recipients met the CDC-ILI definition. Therefore, we were unable to obtain a meaningful estimate of the efficacy of FluBlok against CDC-ILI due to strains represented in the vaccine.

The remaining 111 influenza A viruses were characterized as antigenic variants, i.e., they exhibited a 4-fold or greater decrease in reactivity with post infection ferret antisera in reciprocal HAI testing. These viruses included 12 H1N1 viruses antigenically resembling H1 drift variant A/Brisbane/59/2007, 41 H3N2 viruses antigenically resembling the H3 drift variant A/Brisbane/10/2007, 42 H3N2 viruses that could not be identified as either A/Wisconsin-like or A/Brisbane-like, and 16 influenza viruses for which the subtype was not determined. Fifty-eight of the 59 influenza B viruses (98%) were antigenically similar to B/Florida/04/2006, representing a different clade from the vaccine strain, and one influenza B virus could not be antigenically characterized.

The cumulative rates of culture confirmed influenza illness occurring in the study population over time are shown in Fig. 2, which shows that the relative difference between the two study groups remained constant throughout the influenza season. The incidence of this and of other related endpoints in the study by group are shown in Table 3. The overall protective efficacy of FluBlok against culture positive CDC-ILI regardless of strain was 44.6% (95% CI 18.8%, 62.6%). As expected given the more extreme degree of antigenic mismatch for the influenza B component, the efficacy of FluBlok was greater against CDC-ILI due to influenza A viruses (54.4%) than influenza B viruses (23.1%), although the difference was not statistically significant ($P = .20$ by logistic regression). However, we were not able to demonstrate a statistically significant effect for influenza B. As shown in Table 3, there were no substantial differences between the point estimates of efficacy against culture positive CDC-ILI or against any culture positive illness. Culture positive CDC-ILI associated with isolation of H1N1 viruses occurred in 9/2304 placebo recipients and 3/2344 FluBlok recipients (PE = 67%), while culture positive CDC-ILI due to H3N2 viruses

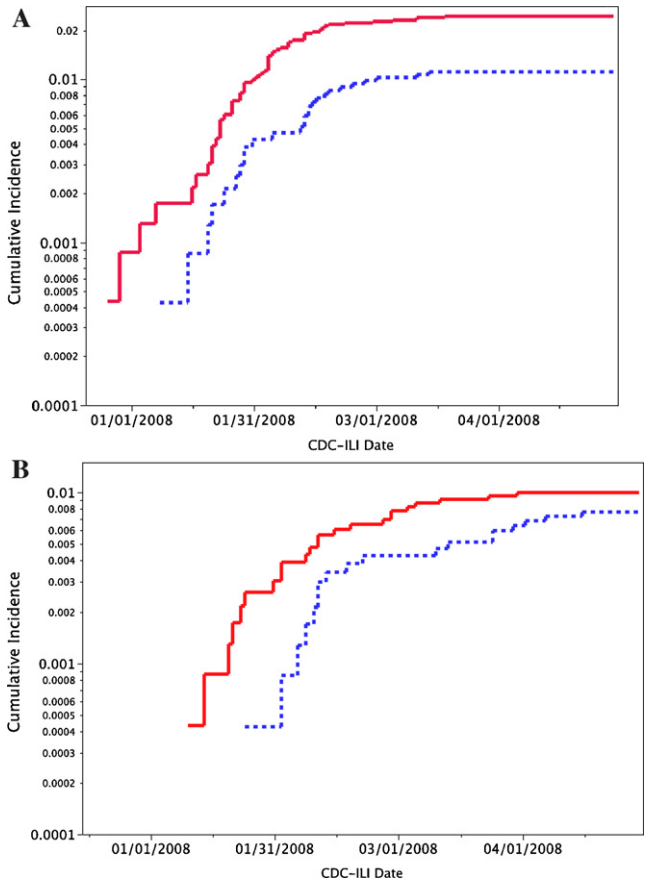

Fig. 2. Kaplan–Meier plots of the cumulative incidence of culture positive CDC ILI due to influenza A (A) and influenza B (B) in the placebo (solid line) and FluBlok (dotted line) groups by date during the surveillance period.

occurred in 58/2304 placebo and in 33/2344 FluBlok recipients (PE = 44%). Overall CDC-ILI regardless of culture results occurred in 162 (7.0%) of placebo recipients and 127 (5.4%) of FluBlok recipients for an efficacy of 22.9% (95% CI, 2.2%, 39.4%) against this outcome.

4. Discussion

In this prospective, randomized clinical trial, we evaluated the safety, immunogenicity and efficacy of FluBlok, a trivalent recombinant hemagglutinin (rHA0) vaccine. The vaccine was well tolerated in healthy adults, immunogenic, and protective against culture confirmed influenza illness. The safety data generated in this study are consistent with the safety profile observed in other studies of rHA0 vaccine produced using the baculovirus expression system in healthy adults [9]. These vaccines have been well tolerated at all doses administered, and are associated with low rates of local reactions. One case of pericarditis was detected in a FluBlok recipient. Pericarditis has also been reported previously following vaccination with licensed, egg-derived influenza vaccine with a similar time course [11]. The mechanism and clinical significance of such an association is unclear.

Table 3
Rates of study endpoints and percent protective efficacy in FluBlok and Placebo recipients.

Endpoint	Number (%) of cases in those receiving		Vaccine efficacy (95% CI)
	FluBlok (N = 2344)	Placebo (N = 2304)	
Influenza positive CDC-ILI[a]			
Any	44 (1.9)	78 (3.4)	44.6 (18.8, 62.6)
Influenza A	26 (1.1)	56 (2.4)	54.4 (26.1, 72.5)
Influenza B	18 (0.8)	23 (1.0)	23.1 (−49.0, 60.9)
Influenza positive illness			
Any	64 (2.7)	114 (4.9)	44.8 (24.4, 60.0)
Influenza A	41 (1.7)	79 (3.4)	49.0 (24.7, 65.9)
Influenza B	23 (1.0)	36 (1.6)	37.2 (-8.9, 64.5)

[a] CDC-ILI was defined as fever > 100 F with either cough or sore throat on the same or consecutive days.

FluBlok induced serum HAI antibody responses to all three vaccine components in the majority of recipients, and the lower limit of the 95% confidence interval for response rate exceeded the US FDA criteria for licensure for all three components (lower limit of the 95% confidence interval for response rate >40% and lower limit of the 95% confidence interval for HAI titer of 1:40 or greater >70%) [12].

Although we did not attempt to verify vaccine histories by review of medical records, significant differences in both the frequency and the magnitude of the HAI response were shown when comparing the immune response between subjects with self-reported previous vaccination. Reduced antibody response rates, decreased post vaccination titers, and decreased antibody secreting cell responses have all been observed previously in adults with prior influenza vaccine compared to those without [13–16]. The exact mechanism of this effect remains uncertain, although it may represent interference with antigen presentation by pre-existing antibody [16].

Consistent with the results of national surveillance in the US during the 2007–2008 season [17], the majority of influenza A viruses detected in this study were H3N2 viruses that represented a substantial antigenic mismatch with the vaccine. However, even under these circumstances, FluBlok had significant protective efficacy against culture-confirmed influenza illness, including those meeting the CDC-ILI case definition. Influenza B viruses isolated in this study and elsewhere in North America during the 2007–2008 season belonged to the Yamagata lineage of influenza B viruses, against which vaccines representing the Victoria lineage would be expected to have little or no efficacy. We also found that the efficacy of FluBlok against CDC-ILI associated with influenza B was substantially lower than that against influenza A.

Comparisons of these results with the results of other assessments of the protective efficacy of influenza vaccines are complicated by differences in methodologies, populations, and antigenic match between vaccine and circulating strains in the specific year that studies are carried out. Two recently published studies have evaluated egg-grown inactivated vaccines in healthy adults using a placebo-controlled design during the 2007–2008 influenza season. In one study, conducted primarily in Europe, the overall efficacy of egg-grown inactivated vaccine against culture confirmed illness was 63%, and the lower 95% CI was 46.7% [18]. The predominant influenza A isolates in that study were H1N1 viruses, which were mostly vaccine-like. In another smaller study done on college campuses in Michigan [19], the protective efficacy of TIV against culture confirmed illness was 73% (95% CI, 51%, 85%). In that study, 90% of influenza isolates were influenza A (H3N2), but the antigenic characterization of isolates was not reported. In other recent randomized trials, the protective efficacy of TIV was 22.3% in the 2005–2006 influenza season predominated by influenza B viruses and with overall low attack rates [20], and 49.3% over two seasons, 2005–2007 with most cases due to antigenically variant viruses [21].

Because of the difficulty in conducting placebo-controlled studies of influenza vaccine especially as the target groups for vaccination have expanded, several recent assessments of influenza vaccine effectiveness have utilized a test-negative, case–control design. Estimates of overall inactivated vaccine protective effectiveness in these studies have ranged from 10% to 70% [22–25] and

are clearly impacted by antigenic differences between vaccine and circulating viruses, with highest levels of effectiveness reported for H1 viruses [25] and lowest levels for influenza B.

The current study provides evidence of protective efficacy of a baculovirus-derived HA vaccine in adults for prevention of seasonal influenza and supports that significant protection in a primed population can be obtained against influenza with a pure hemagglutinin vaccine. The efficacy results also demonstrate that in adults, the minor differences in HA glycosylation seen in insect cells compared to mammalian cells and the synthesis of the HA as an uncleaved precursor do not preclude the generation of an effective immune response in adults.

The use of recombinant DNA techniques to express proteins in cell culture has been a successful approach for generation of highly effective vaccines for the prevention of hepatitis B (HBV) and human papillomavirus (HPV), and baculovirus expression technology is currently used for a licensed HPV vaccine [26]. Among the available expression technologies, recombinant baculovirus is especially well suited for production of influenza vaccine because the rapidity with which genes can be cloned and inserted into this vector facilitates updating the vaccine at regular intervals. Expression of the HA protein in insect cells using recombinant baculovirus also avoids the need to work with potentially pathogenic, live influenza viruses, and the attendant biocontainment issues that would be a particular concern for production of pandemic vaccines [27]. The results of the current study support the utility of insect cells for the production of well tolerated and effective vaccines for seasonal influenza.

Acknowledgements

The authors acknowledge the efforts of Kerry Senger, Tam LaFleur, Anthony Meyer and Adam Gilbert, and the effort of investigators at the study sites listed below.

Study sites: The following investigators and study sites enrolled subjects in this study: Mhorag Hay M.D., University of Rochester School of Medicine, Rochester, NY; Mark Blatter M.D., Primary Physicians Research, Pittsburgh, PA; Stanley Block M.D., Kentucky Pediatric/Adult Research, Bardstown, KY; Bruce Bowling M.D., Regional Clinical Research, Endwell, NY; Teresa Coats M.D., Benchmark Research, Austin, TX; Hana El Sahly M.D., Baylor College of Medicine, Houston, TX; Ivor Emanuel M.D., Benchmark Research, San Francisco, CA; Brandon Essink M.D., Meridian Clinical Research, Omaha, NB; Larry Gilderman D.O., University Clinical Research Pembroke, Pines, FL; Irene Graham M.D., St. Louis University, St. Louis, MO; Matt Hall M.D., Marshfield Clinic, Marshfield, WI; Lydie Hazan M.D., Impact Clinical Trials, Beverly Hills, CA; Darrell Herrington D.O., Benchmark Research, San Angelo, TX; Steven Hull M.D., Vince and Associates Clinical Research, Overland Park, KS; Robert Jeanfreau M.D., Benchmark Research Metairie, LA; James King M.D., University of Maryland, Baltimore, MD; Ivan Rarick M.D., Benchmark Research, Sacramento, CA; Keith Reisinger M.D., Primary Physicians Research, Pittsburgh, PA; Robert Rosen M.D., Carolina Medical Trials, Winston-Salem, NC; Gilbert Schiff M.D., Sterling Research, Cincinnati, OH; William Seger M.D., Benchmark Research Fort, Worth, TX; Helen Keipp Talbot M.D., Vanderbilt University, Nashville, TN; Gilbert Podolsky M.D., Jean Brown Research Salt, Lake City, UT; Birgit Winther M.D., University of Virginia, Charlottesville, VA.

Conflict of interest statement: J.T. has received grant support for clinical trials from Protein Sciences Corporation, Wyeth, Sanofi, GlaxoSmithKline, PaxVax, Ligocyte, and Vaxinnate, and is a paid consultant to ITS incorporated and Novartis. H.E.-S. has received grant support from GlaxoSmithKline for clinical trials. I.G., and J.K. report no conflicts of interest. P.P. and R.K. are paid consultants to Protein Sciences and multiple other entities. R.I. and M.C. are employees of Protein Sciences. *Financial support*: The study was funded by Protein Sciences Corporation, the manufacturers of the vaccine. Protein Sciences Corporation also funded ICON as the CRO for the study and Ockham which performed the data analysis.

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