AS03$_B$-Adjuvanted H5N1 Influenza Vaccine in Children 6 Months Through 17 Years of Age: A Phase 2/3 Randomized, Placebo-Controlled, Observer-Blinded Trial

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Background. This phase 2/3, randomized, placebo-controlled, observer-blinded study assessed the immunogenicity, reactogenicity, and safety of an inactivated, split-virion H5N1 influenza vaccine (A/Indonesia/5/2005) in children aged 6 months through 17 years.

Methods. Children received 2 influenza vaccine doses 21 days apart, each containing 1.9 µg of hemagglutinin and AS03$_B$ adjuvant (5.93 mg of α-tocopherol). The randomization ratio was 8:3 for vaccine to placebo, with equal allocation between 3 age strata (6–35 months, 3–8 years, and 9–17 years). Immunogenicity against the vaccine strain was assessed 21 days after the first and second vaccine doses for all vaccinees, at day 182 for half, and at day 385 for the remaining half. Reactogenicity after each dose and safety up to 1 year after vaccination were evaluated.

Results. Within each age stratum, the lower limit of the 98.3% confidence interval for the day 42 seroprotection rate was ≥70%, thus fulfilling the US and European licensure criteria. The immune responses elicited by vaccine persisted well above baseline levels for 1 year. The vaccine was more reactogenic than placebo, but no major safety concerns were identified.

Conclusions. AS03$_B$-adjuvanted H5N1 influenza vaccine was immunogenic and showed an acceptable safety profile in all age groups studied.

Clinical Trials Registration. NCT01310413.

Keywords. AS03$_B$; H5N1; influenza vaccine; *Prepandrix*™; pandemic; adjuvant.

From 2003 through January 2014, the World Health Organization (WHO) reported 650 cases of avian influenza A(H5N1) infection in humans, of which 386 (59%) were fatal [1]. Children and adolescents are particularly vulnerable to complications associated with novel influenza viruses. In a study including 193 children (age, <18 years) with confirmed H5N1 infection from 13 countries, the case-fatality rate was 48.7% [2]. Additionally, during the 2009 pandemic of swine-origin influenza A(H1N1) rates of infection with influenza virus and associated hospitalizations among children (age, <18 years) were higher than

those among elderly individuals (age, ≥65 years) [3, 4]. The pandemic potential of novel influenza viruses should not be underestimated, and the continued development of vaccines against avian-origin H5N1 influenza viruses remains a public health priority.

There are 2 H5N1 vaccines currently licensed in the United States, manufactured by Sanofi-Pasteur and GlaxoSmithKline Vaccines, and various H5N1 vaccines are licensed in Europe [5, 6]. Of those licensed in Europe, GlaxoSmithKline has produced H5N1 vaccines containing the A/Indonesia or the A/Vietnam antigen and formulated with the oil-in-water adjuvant system AS03 (Prepandrix™ [GlaxoSmithKline, Dresden, Germany]; Adjupanrix™ [GlaxoSmithKline, Dresden, Germany], and Pumarix™ [GlaxoSmithKline, Québec, Canada]). These vaccines have been shown to elicit strong, sustainable, and cross-clade immune response in adults [7, 8].

H5N1 influenza vaccine (the antigen for which is produced in Dresden) has also been evaluated in a phase 2, randomized, open-label study in children aged 3–9 years, which showed that 2 doses of vaccine containing 1.9 μg or 3.75 μg of H5N1 hemagglutinin antigen (HA) and adjuvanted with 2 different AS03 dosages, referred to as AS03$_A$ (11.86 mg of tocopherol) and AS03$_B$ (5.93 mg of tocopherol), elicited strong antibody responses against the vaccine and drifted strains [9].

In this study, immunogenicity and antibody persistence up to 1 year after vaccination with H5N1 influenza vaccine (AS03$_B$-adjuvanted H5N1 A/Indonesia/5/2005 with antigen produced in Québec) in children aged 6 months through 17 years was evaluated. Reactogenicity and safety were also assessed.

METHODS

This phase 2/3 randomized, placebo-controlled, observer-blinded trial evaluated the immunogenicity, reactogenicity, and safety of a 2-dose primary vaccination series of AS03$_B$-adjuvanted H5N1 A/Indonesia/5/2005 vaccine in children (clinical trials registration NCT01310413). The study was conducted in the United States, Canada, and Thailand. Healthy children aged 6 months through 17 years at the time of first vaccination were included. Exclusion criteria included previous receipt of H5N1 vaccine; receipt of seasonal influenza vaccine within 14 days (inactivated vaccine) or 30 days (live vaccine); receipt of any vaccine not foreseen by the protocol up to 42 days from baseline; receipt of any investigational or nonregistered product from 30 days before to 42 days after study vaccination; any significant acute or chronic uncontrolled illness; temperature of ≥38°C (≥100.4°F) at baseline assessment; cancer diagnosis within previous 3 years; immunosuppressive or immunodeficient conditions; receipt of glucocorticoids within 1 month of the start of and throughout the study; receipt of cytotoxic, immunosuppressive drugs within 6 months of the start of and throughout the study; receipt of immunoglobulins within

3 months of the start of and throughout the study; and history of allergy to influenza vaccine.

All protocols and study documents were approved by independent/local ethics committees in accordance with good clinical practice, the Declaration of Helsinki, and regulatory requirements. Parents/guardians provided informed written consent, and children 9 to <18 years of age provided assent according to local standards.

Vaccines and Randomization

The study vaccine was an inactivated, split-virion H5N1 influenza vaccine manufactured by GlaxoSmithKline in Québec, Canada. It contained 1.9 μg of HA from H5N1 A/Indonesia/5/2005 adjuvanted with AS03$_B$, a GlaxoSmithKline proprietary adjuvant system containing α-tocopherol and squalene in oil-in-water emulsion (5.93 mg of tocopherol). The antigen component and the adjuvant were mixed at a ratio of 1:1, resulting in a final volume of 0.25 mL per dose. The placebo was 0.25 mL of saline.

Randomization was performed using a blocking scheme, and treatment allocation at study sites was done using an Internet-based system. Children were randomized at a ratio of 8 to 3 to receive H5N1 vaccine or placebo, respectively, with equal allocation between 3 age strata (6–35 months, 3–8 years, and 9–17 years).

Vaccine or placebo injections were administered in the non-dominant (dose 1) and dominant arm (dose 2) 21 days apart. Participants and study personnel involved in the collection and analysis of data were blinded to treatment.

Immunogenicity Assessments

Immunogenicity was assessed for all children at days 0, 21, and 42; for half of the children in each age strata at day 182; and for the other half at day 385.

Hemagglutination inhibition (HI) assays were performed using an established method, modified for equine rather than avian erythrocytes [10–13]. HI antibody parameters were geometric mean titer (GMT), seroprotection rate (SPR; defined as the percentage of children with HI titers of ≥1:40 following vaccination), seroconversion rate (SCR; defined as the percentage of children achieving an increase in HI titers from <1:10 to ≥1:40 or at least a 4-fold postvaccination increase in HI titer from a prevaccination titer of ≥1:10), and mean geometric increase (MGI; defined as the geometric mean of the ratio of postvaccination to prevaccination reciprocal HI titers). Subjects with HI antibody titers of ≥1:10 were considered to be seropositive.

Virus neutralizing antibody (nAb) titers were determined using a microneutralization assay described previously [10, 12]. The 50% neutralization titer of serum was calculated by use of the method of Reed and Muench [14]. The assay cutoff titer was 1:28. A nAb response was defined as a minimum 4-fold increase from the prevaccination reciprocal titer (titers of <28 were assigned a value of 14); the vaccine response rate was

the percentage of subjects making a response. Assessment of nAbs was a secondary objective performed on randomly selected subsets of 40 subjects for each age stratum in the study vaccine group and 10 subjects per age stratum in the placebo group for homologous and drift variant assays. The same subjects were to be analyzed for homologous and heterologous strains at day 0 and day 42. Separate subsets of the same numbers of subjects per group were evaluated at day 182 and day 385.

Reactogenicity and Safety Assessments

Solicited local symptoms (ie, those at the injection site) and general symptoms were assessed during the 7-day postvaccination period after each dose of H5N1 vaccine or placebo. Parents/guardians recorded the occurrence and severity of solicited events on diary cards. Local symptoms were pain, redness, and swelling. General symptoms were drowsiness, irritability/fussiness, loss of appetite, and fever, for children aged <6 years; and fatigue, fever, gastrointestinal symptoms, headache, joint pain, muscle aches, shivering, and sweating, for children aged ≥6 years. Fever was defined as a temperature of ≥38.0°C (100.4°F) by any route or method [15]. All solicited local events were considered to be vaccine-related, whereas investigators provided causality assessments for solicited general events.

All unsolicited adverse events (AEs) were recorded from day 0 to day 42 after each dose. Serious AEs (SAEs), medically attended AEs (MAEs), and potential immune-mediated diseases (pIMDs) were recorded from day 0 to day 385 (Supplementary Table 1). SAEs were defined as events that resulted in death, hospitalization, or disability/incapacity or were life threatening. MAEs were defined as events for which the child was hospitalized, visited the emergency department, or had a visit from a physician for any reason. pIMDs were a subset of AEs including autoimmune diseases and other inflammatory and/or neurologic disorders that may have an autoimmune etiology. Unsolicited events were coded using the *Medical Dictionary for Regulatory Activities*, and investigators provided causality assessments for unsolicited AEs, SAEs, MAEs, and pIMDs. Serum chemistry findings and hematological findings were assessed for all
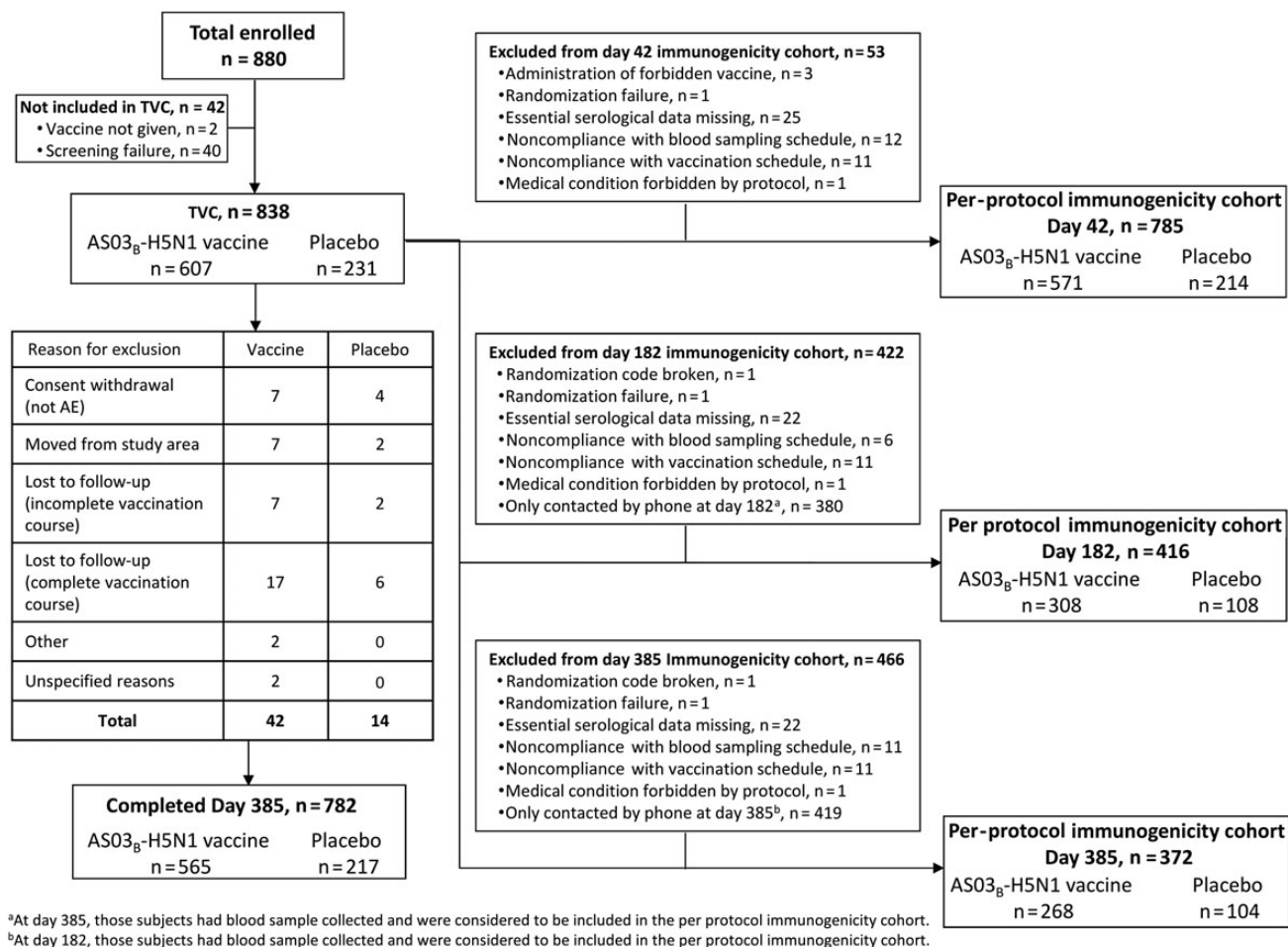
Figure 1. Selection and flow of subjects through the study. Abbreviations: AE, adverse event; TVC, total vaccinated cohort.

Table 1. Demographic Characteristics in the Total Vaccinated Cohort, by Age Stratum at Vaccination

Characteristic	AS03$_B$-H5N1 Group (n = 607)			Placebo Group (n = 231)		
	6–35 mo	3–8 y	9–17 y	6–35 mo	3–8 y	9–17 y
Age, mo						
Mean ± SD	21.7 ± 8.2	70.5 ± 21.7	160.8 ± 28.2	22.6 ± 8.2	65.2 ± 20.2	156.0 ± 31.3
Median	23.0	71.5	160.0	24.0	60.0	155.5
Male sex, no. (%)	107 (53.8)	108 (54.5)	107 (51.0)	36 (48.0)	41 (53.9)	38 (47.5)
Ethnic origin, no. (%)						
White Caucasian/European heritage	77 (38.7)	89 (44.9)	105 (50.0)	26 (34.7)	37 (48.7)	43 (53.8)
Asian/South-East Asian heritage	77 (38.7)	68 (34.3)	66 (31.4)	30 (40.0)	26 (34.2)	27 (33.8)
Other[a]	45 (22.6)	41 (20.7)	39 (18.6)	19 (25.3)	13 (17.1)	10 (12.5)

[a] American Indian or Alaskan native, Asian/Central/South Asian heritage, Asian/East Asian heritage, White/Arabic/North African Heritage, African American, or other.

patients at days 0 and 42, for half of patients at day 182, and for the remaining half at day 385 (Supplementary Table 2).

Objectives

The primary objective was to evaluate HI antibody titers against the vaccine strain 21 days after the second vaccination (day 42) for the H5N1 vaccine group stratified by age. The success criterion was to demonstrate that the lower limit of the 98.3% confidence interval (CI) for the SPR was ≥70% for each age stratum at day 42, thus meeting or exceeding a US Center for Biologics Evaluation and Research (CBER) immunogenicity licensure criterion for adults and children and a European Committee for Medicinal Products for Human Use (CHMP) licensure criterion for adults [16, 17].

Secondary immunogenicity objectives were to assess HI antibody responses (GMT, SPR, SCR, and MGI) stratified by age against the vaccine strain at days 0, 21, 42, 182, and 385; to evaluate whether day 42 responses fulfilled CBER and CHMP immunogenicity licensure criteria; and to describe nAb responses against the vaccine strain and a drifted strain stratified by age at days 0, 21, 42, 182, and 385.

Secondary safety objectives were to assess solicited local symptoms and general symptoms during the 7-day postvaccination period; unsolicited AEs from day 0 to day 42; and SAEs, MAEs, and pIMDs from day 0 to day 385. Grade 3 AEs were defined as AEs that prevented normal, everyday activities.

Analyses

Target enrollment was 825 children (600 in the vaccine group and 225 in the placebo group), to provide 83.3% power to demonstrate that the lower limit of the 98.3% CI for SPR would be ≥70% in each of 3 age strata at day 42, using a 2-sided 1-proportion test ($\alpha = 1.67\%$) and assuming that 20% of subjects would be unevaluable (PASS 2005, NCSS Statistical Software, UT).

Secondary HI immunogenicity outcomes were tabulated with 95% CIs. To fulfill CBER licensure criteria, the lower limit of the 95% CIs for SCR needed to be ≥40%; and to fulfill CHMP criteria, point estimates for SCR needed to be >40%; for SPR, >70%; and for MGI, >2.5. nAb responses were tabulated with 95% CIs. Immunogenicity was described in the per-protocol immunogenicity cohort that included children who met the eligibility criteria, who complied with the protocol, and for whom data were available at the specified evaluation time point.

AEs, SAEs, and MAEs were analyzed descriptively and tabulated with 95% CIs. Reactogenicity and safety were assessed in the total vaccinated cohort, which comprised children who received at least 1 dose of vaccine or placebo.

RESULTS

A total of 838 children were vaccinated, including 607 in the H5N1 vaccine group and 231 in the placebo group (Figure 1). For the total vaccinated cohort, the country distribution of enrolled subjects included 96 in Canada, 292 in Thailand, and 450 in the United States. An overview of the study cohorts and reasons for withdrawal is shown in Figure 1. The study groups were balanced for demographic characteristics at baseline (Table 1). The first child was enrolled on 7 March 2011, and the final contact (day 385) was on 4 July 2012. The safety database was closed on 13 September 2012.
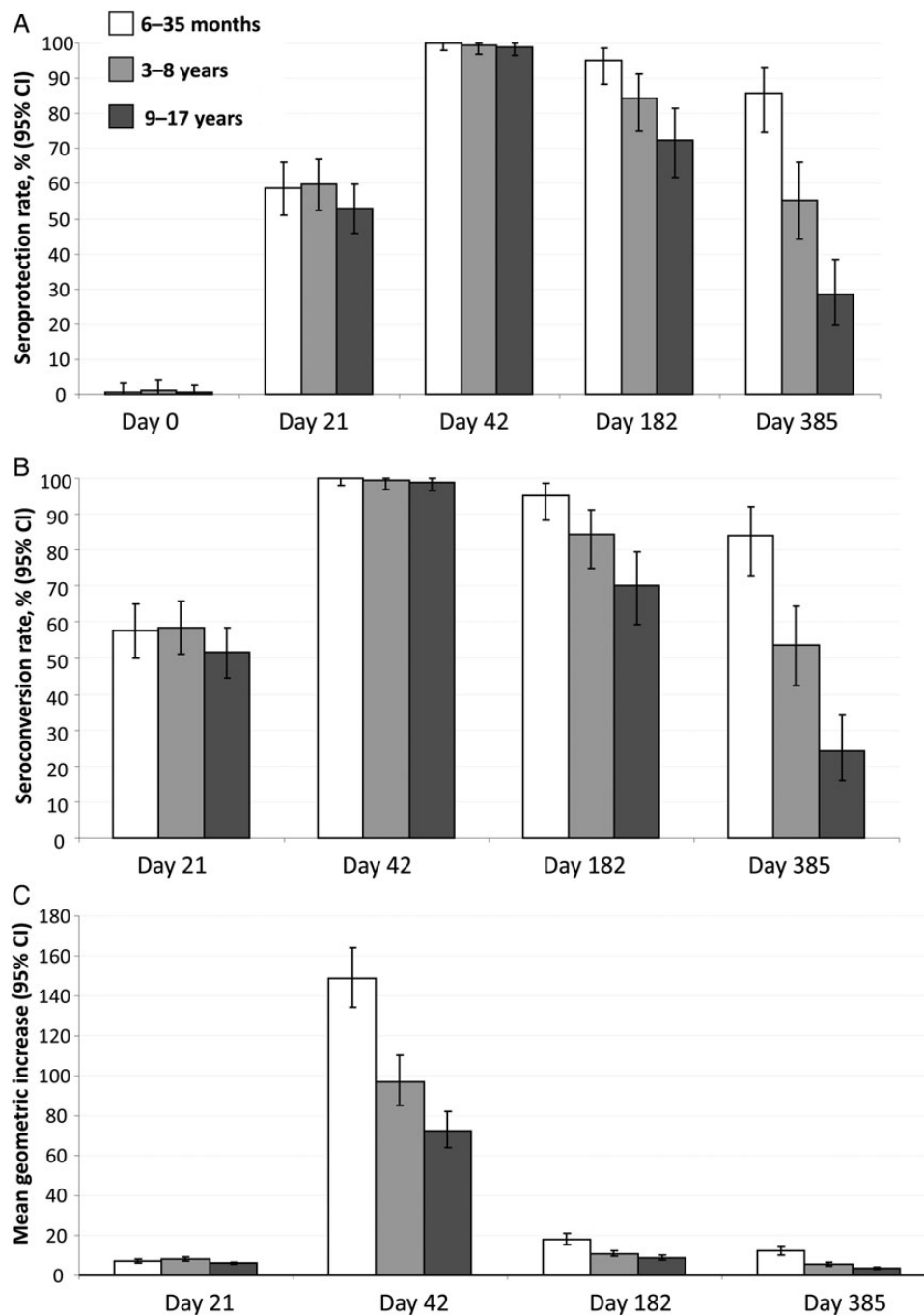
Table 2. Hemagglutination Inhibition (HI) Seroprotection Rates (SPRs) for the AS03B-H5N1 Vaccine Group at Day 42 in the Per-Protocol Immunogenicity Cohort, by Age Stratum at Vaccination

Age Stratum	SPR,[a] % (98.3% CI)
6–35 mo (n = 175)	100 (97.3–100)
3–8 y (n = 185)	99.5 (96.4–100)
9–17 y (n = 203)	99.0 (95.8–99.9)

Data for the placebo group are not shown.

Abbreviation: CI, confidence interval.

[a] Defined as the proportion of children with HI antibody titers of ≥1:40. The licensure threshold is defined as lower limit of ≥70% for the 95% CI (by the US Center for Biologics Evaluation and Research) and as a point estimate of ≥70% (by the European Committee for Medicinal Products for Human Use).

Figure 2. Hemagglutination inhibition (HI) seroprotection rates (*A*), seroconversion rates (*B*), and mean geometric increase (*C*) in the per-protocol immunogenicity cohort, by age stratum. Seroprotection rate was defined as the percentage of children with HI titers of ≥1:40 following vaccination; seroconversion rate was defined as the percentage of children achieving an increase in HI titer from <1:10 to ≥1:40 or at least a 4-fold postvaccination increase in HI titer from a prevaccination titer of ≥1:10. Abbreviation: CI, confidence interval.

Immunogenicity

HI Antibody Responses Against A/Indonesia in the AS03$_B$-H5N1 Vaccine Group

Within each age stratum, the lower limit of the 98.3% CI for the day 42 SPR was ≥70%, meeting the primary objective (Table 2). At day 42, all CBER and CHMP immunogenicity licensure

criteria were met for each age stratum. SPRs and SCRs were each >99.0% for all age strata (Figure 2).

Overall, 9.8% of subjects were seropositive before vaccination. Prevaccination seropositivity rates were 5.5%, 10.9%, and 12.7% in the groups aged 6–35 months, 3–8 years, and 9–17 years, respectively. Four subjects had HI titers of ≥1:40 before

vaccination; 1 each in the groups aged 6–35 months and 9–17 years and 2 in the group aged 3–8 years. At day 21, GMTs ranged from 35.3 in the group aged 9–17 years to 44.6 in the group aged 3–8 years (Table 3). At day 42, there was a 10–20-fold increase in GMTs, with the greatest increase among children aged 6–35 months (Table 3). At day 385, the seropositivity rate was >94.7%, and GMTs were 4–12-fold greater than those before vaccination (Table 3). At days 182 and 385, SPRs were 72.4%–95.2% and 28.4%–85.7%, respectively (Figure 2A). No differences were observed in GMTs when segregated according to country. No HI responses were observed with placebo (data not shown).

nAb Responses in the AS03$_B$-H5N1 Vaccine Group

The seropositivity rates and GMTs for nAb against the vaccine strain (clade 2.1, A/Indonesia/5/05) and the drifted strain (clade 1, A/Vietnam/1194/2004) at days 42, 182, and 385, segregated according to age groups, are shown in Supplementary Table 3. For both strains, the nAb response peaked at day 42 and generally decreased over time. GMTs at day 385 remained >5 times above baseline for the vaccine strain and >2 times above baseline for the drifted strain. At day 385, the majority of children remained seropositive for both strains.

The vaccine response rates against the vaccine strain at day 42 in the groups aged 6–35 months, 3–8 years, and 9–17 years were 100%, 100%, and 97.5%, respectively, and response rates against the drifted strain were 88.2%, 72.2%, and 40.0%, respectively. No nAb responses were observed with placebo (data not shown).

Safety

Reactogenicity

The frequency of local and general symptoms, stratified by age, are shown in Figure 3. The frequencies of any local symptom during the 7-day periods after the first and second doses were similar, at 58.8% and 51.4%, respectively, in the H5N1 vaccine group, and 23.4% and 19.6%, respectively, in the placebo group. This was also observed for the frequency of any general symptoms during the 7-day periods after the first and second doses, at 48.1% and 42.0%, respectively, in the H5N1 vaccine group, and 37.2% and 33.5%, respectively, in the placebo group.

The frequency of solicited local symptoms in the H5N1 vaccine group was higher than that in the placebo group. Overall (ie, per subject rather than per dose), pain at the injection site was the most frequent local symptom, with cases observed in 67.2% and 30.1% of children in the H5N1 vaccine and placebo groups, respectively, of which 4.1% and 1.7%, respectively, experienced grade 3 pain.

In children aged <6 years, overall (ie, per subject), irritability/fussiness and drowsiness were the most common general symptoms, at 43.5% and 34.4%, respectively, in the H5N1 group, and 32.8% and 23.8%, respectively, in the placebo group. In children aged 6–17 years, muscle aches, headache, and fatigue were the

Table 3. Hemagglutination Inhibition Geometric Mean Titers (GMTs) in the AS03$_B$-H5N1 Vaccine Group in the Per-Protocol Immunogenicity Cohorts, by Age Stratum at Vaccination

Age Stratum, Day	Subjects, No.	GMT (95% CI)
6–35 mo		
0	182	5.3 (5.1–5.5)
21[a]	179	38.7 (33.9–44.2)
42[b]	175	777.1 (705.6–855.9)
182	84	90.6 (78.1–105.0)
385	63	65.6 (55.9–76.9)
3–8 y		
0	184	5.6 (5.3–5.9)
21[a]	184	44.6 (39.2–50.9)
42[b]	185	543.8 (484.9–609.8)
182	89	57.4 (50.8–64.9)
385	85	32.8 (28.1–38.4)
9–17 y		
0	204	5.7 (5.4–6.1)
21[a]	204	35.3 (31.7–39.5)
42[b]	203	416.2 (371.5–466.2)
182	87	50.2 (43.3–58.2)
385	95	21.6 (18.6–25.1)

Immunogenicity was assessed in all children at days 0, 21, and 42; in half of the children at day 182; and in the remaining half at day 385. Data for the placebo group are not shown.

Abbreviation: CI, confidence interval.

[a] Twenty-one days after dose 1.

[b] Twenty-one days after dose 2.

most frequent general symptoms in both groups: 39.8% and 15.9% of children in the H5N1 vaccine and placebo groups, respectively, had muscle aches; 32.4% and 16.8%, respectively, had headache; and 28.8% and 17.8%, respectively, had fatigue. Other general solicited symptoms were observed in <20% of children.

The rates of fever (temperature, ≥38°C [≥100.4°F]) and grade 3 fever (≥39°C [≥102.2°F]) in the group aged 6–35 months were 22.4% and 4.6%, respectively, for the H5N1 group and 16.4% and 5.5%, respectively, for the placebo group. For children aged 3–5 years, these rates were 15.3% and 5.1%, respectively, for the H5N1 group and 18.4% and 2.0%, respectively, for the placebo group. For the group aged 6–8 years, these rates were 13.1% and 4.0%, respectively, for the H5N1 group, with no reports of fever in the placebo group. In the group aged 9–17 years, the rates were 2.9% and 0.5%, respectively, for the H5N1 group and 3.8% and 1.3%, respectively, for the placebo group. Up to day 42, 57.3% of children overall in the H5N1 group received any antipyretic treatment, compared with 48.9% in the placebo group.

Unsolicited AEs

There were no withdrawals from the study due to an AE or SAE. A summary of unsolicited AEs is shown in Table 4.
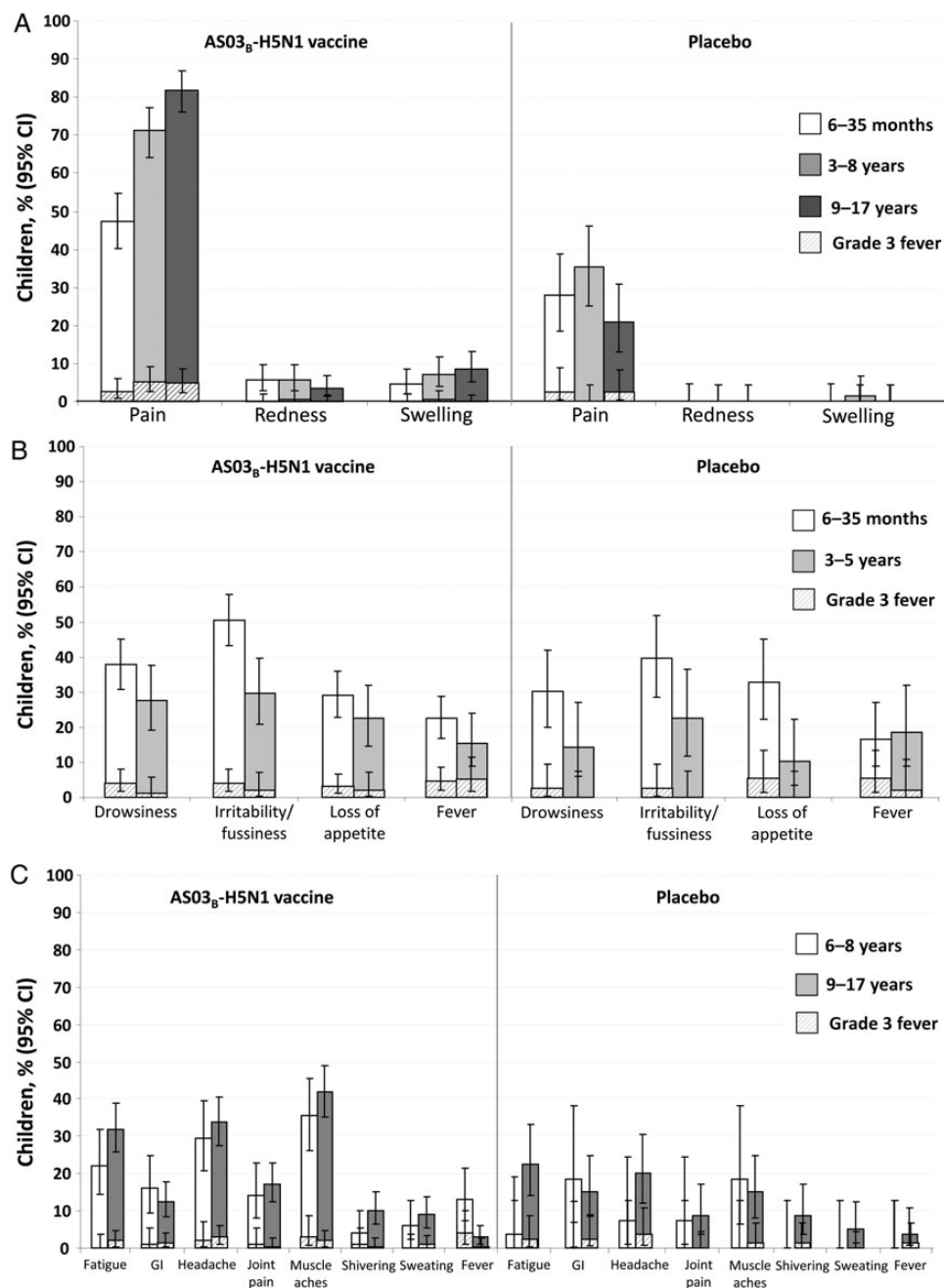
Figure 3. Solicited injection site events (*A*) and general solicited adverse events (*B* and *C*) in the total vaccinated cohort, by age stratum. Grade 3 fever was defined as a temperature of ≥39°C (≥102.2°F). Data are shown as events/child. Abbreviations: CI, confidence interval; GI, gastrointestinal.

Up to day 385, the proportion of children experiencing at least 1 MAE was similar between the H5N1 vaccine group (189 of 607 [31.1%]) and the placebo group (77 of 231 [33.3%]); the most common MAEs were upper respiratory tract infection, cough, otitis media, and pharyngitis. In the H5N1 vaccine group, SAEs were dehydration, inguinal hernia, infectious mononucleosis, influenza and pneumonia, upper respiratory tract infection, skeletal injury, febrile convulsion, spontaneous abortion, and bronchial hyperactivity; SAEs in the placebo group were lymphadenitis, type 1 diabetes, suicidal ideation, and asthma. The case of febrile convulsion (grade 2) occurred in a 30-month-old boy 11 days after the first dose of vaccine, which resolved after 3 days and was not considered by the investigator to be vaccine related.

Up to day 385, there were 2 pIMDs: alopecia in the H5N1 vaccine group, which was not considered by the investigator as vaccine related, and type 1 diabetes in the placebo group, which was reported to be related to vaccination before unblinding.

Table 4. Summary of Unsolicited Adverse Events (AEs) in the Total Vaccinated Cohort, by Follow-up Day

Follow-up Day	AS03$_B$-H5N1 Group, Subjects, No. (%) (n = 607)	Placebo Group, Subjects, No. (%) (n = 231)
Days 0–42		
≥1 AE(s)	244 (40.2)	98 (42.4)
≥1 grade 3 AE(s)	22 (3.6)	10 (4.3)
≥1 AE(s) causally related to vaccination	38 (6.3)	7 (3.0)
Days 0–385		
≥1 MAE(s)	189 (31.1)	77 (33.3)
≥1 SAE(s)	8 (1.3)	4 (1.7)
≥1 pIMD(s)	1 (0.2)	1 (0.4)

Abbreviations: MAE, medically attended AE; pIMD, potential immune-mediated diseases; SAE, serious AE.

Serum chemistry findings and hematological results did not reveal differences between the groups (data not shown).

DISCUSSION

Our study showed that 2 doses of influenza vaccine containing 1.9 μg of HA from H5N1 A/Indonesia/5/2005 adjuvanted with AS03$_B$ (manufactured by GlaxoSmithKline in Québec, Canada) was immunogenic in children 6 months through 17 years of age. HI antibody responses against the vaccine strain fulfilled US and European immunogenicity licensure criteria 21 days after the second vaccination [16, 17]. Immune responses persisted for 1 year after vaccination. Although the vaccine was associated with a higher rate of injection-site and general solicited symptoms than placebo, no safety concerns emerged during the study, and the rate of SAEs up to 1 year after vaccination was 1.3% in the H5N1 vaccine group and 1.7% in the placebo group.

A previous phase 2, open-label study of AS03-adjuvanted H5N1 vaccine (manufactured by GlaxoSmithKline in Dresden, Germany) in children aged 3–9 years described the immunogenicity and safety of the full dose of HA (3.75 μg) adjuvanted with AS03$_A$ (11.86 mg of tocopherol), the full dose of HA adjuvanted with AS03$_B$ (5.93 mg of tocopherol), and a half dose of HA (1.9 μg of HA) adjuvanted with AS03$_B$ [9]. The study evaluated which formulation provided the best balance of immunogenicity, reactogenicity, and safety. It showed that, in children aged 3–5 years and those aged 6–9 years, HI responses against the vaccine-homologous strain (A/Vietnam/1194/2004) fulfilled CBER licensure criteria with all 3 formulations, whereas local events in the half-dose formulation tended to be lower than those in the other formulations [9]. In our study, we evaluated 2 doses of vaccine that contained 1.9 μg of H5N1 HA adjuvanted with AS03$_B$ but were made using a different production process and strain, A/Indonesia/5/2005, to expand the clinical experience with this type of vaccine in children as young as 6 months and as old as 17 years.

We observed robust HI antibody responses after 2 doses of vaccine containing 1.9 μg of H5N1 HA adjuvanted with AS03$_B$, thus confirming the antigen-sparing potential of the vaccine. Before vaccination, 9.8% of children were seropositive against the vaccine strain, and after vaccination, HI antibody responses fulfilled licensure criteria for immunogenicity, with >99.0% of children achieving titers of ≥1:40. Interestingly, a modest age-dependent decrease in GMT was observed, with lower responses with increasing age. The unexpected strength of the immune response in the youngest children suggests that those with the least previous exposure to influenza A virus are able to mount the greatest HI antibody response to the adjuvanted vaccine. Following H5N1 influenza vaccination, low post-vaccination antibody titers have been reported in subjects who had previously received seasonal influenza vaccination, compared with those who had not [18–21]. Preexisting antibodies resulting from seasonal influenza virus infection or vaccination are suspected of having a negative effect on the immune response elicited by subsequent doses of influenza vaccine [22]. Also, it has been hypothesized that previous vaccination with a nonadjuvanted seasonal influenza vaccine might lead to the shifting of the T-cell and/or B-cell repertoires toward epitopes specific for the antecedent vaccine, thus affecting the ability of immune system to evoke an immune response against subsequent vaccination and resulting in lower HI titers [21].

HI antibodies persisted for 1 year after vaccination in the vast majority of children, although some age-related differences in persistence were observed. In children aged 6–35 months, HI antibody responses were highest at day 42 and also at 1 year after vaccination. In the 2 older age strata, there was an incremental decrease in HI antibody levels both at day 42 and 1 year. Nevertheless, the dose evaluated in this study offered impressive immunogenicity to children regardless of age.

nAb responses were high in all age strata, with ≥97.5% of children achieving a minimum 4-fold increase from the prevaccination reciprocal titer at day 42. At day 385, the seropositivity rate was >94.6%, and GMTs were 5–12-fold greater than those before vaccination. In addition to responses against the vaccine strain, A/Indonesia/5/2005, neutralizing responses were observed against a drifted strain, A/Vietnam/1194/2004 (which is consistent with previous reports of AS03-H5N1 vaccines in adults and children, which showed strong cross-reactivity [9, 23].

As expected, we observed a higher rate of local symptoms and general symptoms in the vaccine group, compared with the placebo group, consistent with previous studies of AS03-adjuvanted vaccines in children and adults [9, 24].

Recent reports suggested a possible link between the 2009 pandemic influenza A(H1N1) strain vaccine (which is distinct from the strain we used) and subsequent onset of narcolepsy

[25–35]. Further investigations will help elucidate the chain of events that resulted in narcolepsy and the potential roles of genetic and environmental factors in triggering narcolepsy. No cases of narcolepsy were recorded in this study, which was not designed to detect rare events such as narcolepsy.

To conclude, the potential threat posed by avian-origin H5N1 influenza viruses should not be underestimated, and the development of vaccines suitable for use in vulnerable groups such as children is a public health priority. In this study, we showed that 2 doses of vaccine containing 1.9 µg of H5N1 HA adjuvanted with $AS03_B$ provided robust immune responses in children aged 6 months to 17 years, with >94.6% remaining seropositive for vaccine-homologous nAb for at least 1 year. $AS03_B$-H5N1 vaccine was more reactogenic than placebo, but overall no major safety concerns were identified.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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