Safety and Long-term Humoral Immune Response in Adults After Vaccination With an H1N1 2009 Pandemic Influenza Vaccine With or Without AS03 Adjuvant

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(See the editorial commentary by Neuzil, on pages 700–1.)

Background. In this study (NCT00985088) we evaluated different formulations of an H1N1 2009 pandemic influenza vaccine that deliver various viral hemagglutinin (HA) doses with or without AS03 (a tocopherol-based oil-in-water adjuvant system).

Methods. A total of 1340 healthy subjects aged ≥18 years were randomized to receive 1 or 2 doses of an adjuvanted (3.75-μg HA/AS03$_A$ or 1.9-μg HA/AS03$_B$) or nonadjuvanted vaccine formulation. Safety and immunogenicity (by hemagglutination-inhibition [HI] assay) after each dose and 6 months after dose 1 are reported here.

Results. A single dose of AS03$_A$-adjuvanted 3.75-μg HA H1N1 2009 induced the strongest immune responses in subjects aged 18−64 years (seroprotection rate [SPR], 97.2%; seroconversion rate [SCR], 90.1%) as well as in subjects aged >64 years (SPR, 91.1%; SCR, 78.2%) 21 days after vaccination. Six months after dose 1, subjects who received 2 doses of either the adjuvanted formulation or 1 dose of the adjuvanted 3.75-μg HA formulation continued to meet all Center for Biologics Evaluation and Research and Committee for Medicinal Products for Human Use criteria. All formulations had clinically acceptable safety profiles.

Conclusion. A single dose of the 3.75-μg HA AS03$_A$-adjuvanted H1N1 2009 influenza vaccine was highly immunogenic in both age strata (18−64 and >64 years), inducing long-term persistence of the immune response until at least 6 months after dose 1.

Mass immunization can be an effective intervention against an influenza pandemic [1]. Anticipating the potential need to protect against heterologous "drifted" strains of virus, the World Health Organization supported the use of adjuvants in pandemic A/California/7/09 H1N1 vaccines in parallel with nonadjuvanted vaccines [2, 3]. Based on previous experience with a (pre)pandemic H5N1 influenza vaccine (3.75-μg hemagglutinin [HA]) adjuvanted with AS03 (a tocopherol-based oil-in-water emulsion adjuvant system) [4–6], GlaxoSmithKline Biologicals (GSK Biologicals) developed an AS03-adjuvanted H1N1 2009 pandemic influenza vaccine with 3.75 μg of HA content. This report presents the findings from a study in adults (≥18 years), including elderly adults (≥64 years), that evaluated whether the humoral immune response induced by this H1N1 2009 pandemic influenza vaccine containing 3.75 μg of HA per dose adjuvanted with AS03$_A$ (11.86 mg of tocopherol) or 1.9 μg of HA adjuvanted with AS03$_B$ (5.93 mg of tocopherol) met the US and European regulatory guidance criteria for evaluation of pandemic influenza vaccines. The study also evaluated the immune responses to nonadjuvanted H1N1 2009 vaccine formulations, the possible effect of added

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adjuvant, and the persistence of immune response until 6 months after the first vaccine dose.

MATERIALS AND METHODS

Study Vaccines

The H1N1 2009 study vaccine was a monovalent, inactivated, split-virion H1N1 2009 pandemic influenza antigen adjuvanted with AS03 (*Arepanrix*™; GSK Biologicals). The H1N1 2009 pandemic vaccine antigen was manufactured using essentially the same procedures as used with the seasonal influenza vaccines *FluLaval*™ and *Fluviral*®.

The H1N1 viral seed for the vaccine was prepared from the reassortant virus NYMC X-179A (New York Medical College, NY) generated from the A/California/07/2009 strain, as recommended by the World Health Organization [2], and propagated in embryonated hen eggs. The antigen suspensions were manufactured to contain 15- or 30-μg/mL H1N1 2009 HA antigen. $AS03_A$/$AS03_B$-adjuvanted vaccine formulations (3.75-μg/1.9-μg HA/0.5-mL dose) and the nonadjuvanted formulation (3.75-μg HA/0.5-mL dose) were prepared from the 15-μg/mL suspension; the nonadjuvanted 7.5- and 15-μg HA/0.5-mL dose formulations were prepared from the 30-μg/mL suspension. After dose 1, 11 weeks after release, single radial immunodiffusion (SRID) results indicated that the estimated HA antigen contents in the nonadjuvanted 7.5- and 15-μg HA/0.5-mL dose formulations were lower than planned (5.6- and 11-μg HA, respectively). Antigen potency was reassessed by SRID after the last vaccine administration and did not vary compared with the baseline values. In this article, these 2 formulations are referred to by their intended dosages (7.5 and 15 μg). The estimated HA content in the $AS03_A$/$AS03_B$-adjuvanted formulations and in the 3.75-μg HA formulation was as intended. Each 0.5-mL dose of the H1N1 2009 vaccine contained 5 μg of thimerosal as preservative.

$AS03_A$ is composed of squalene (10.69 mg), DL-α-tocopherol (11.86 mg), and polysorbate 80 (4.86 mg). $AS03_B$ contained half these amounts of constituents. The adjuvanted vaccine formulations were prepared by mixing the antigen suspension and adjuvant emulsion before administration. The vaccines were administered as an intramuscular injection in the deltoid muscle.

Study Design and Participants

This phase I/II, observer-blind, randomized study (NCT00985088) was conducted at 10 centers in the United States and 4 centers in Canada. Double-blinding was not possible owing to the difference in appearance of the adjuvanted and nonadjuvanted vaccine formulations. The subjects and study personnel who evaluated the safety and immunogenicity end points were blinded; unblinded study personnel responsible for vaccine preparation and administration were not involved in the evaluation of end points.

Adult subjects aged ≥18 years at the time of the first vaccine dose with a satisfactory baseline medical assessment (stable health status with no exclusionary conditions) were randomized using GSK Biologicals' Internet-based randomization system (minimization procedure accounting for study center, age strata, and status of seasonal influenza vaccination) into 8 study groups to receive 1 or 2 doses of either the adjuvanted formulations (3.75-μg HA/$AS03_A$, 1.9-μg HA/$AS03_B$) or the nonadjuvanted formulations (3.75-, 7.5-, or 15-μg HA) of the H1N1 2009 vaccine antigen (Figure 1). To evaluate the impact of the unexpected loss of potency in the nonadjuvanted 7.5- and 15-μg HA formulations (as per SRID results), an unplanned immunogenicity analysis was performed on the first 299 serum sample pairs available at day 21 using the Committee for Medicinal Products for Human Use (CHMP) guidance criteria. It was observed that only subjects aged >60 years receiving 1 dose of the 7.5-μg nonadjuvanted formulation failed to meet CHMP criteria and were offered an additional dose of the same unadjuvanted vaccine any time after day 42.

Volunteers with a history of physician-diagnosed H1N1 2009 influenza infection or vaccination preceding this study, with confirmed or suspected immunosuppressive or immunodeficient conditions, diagnosed with or undergoing treatment for cancer, and/or with a history of allergic or anaphylactic reactions after previous influenza vaccinations were excluded from enrollment.

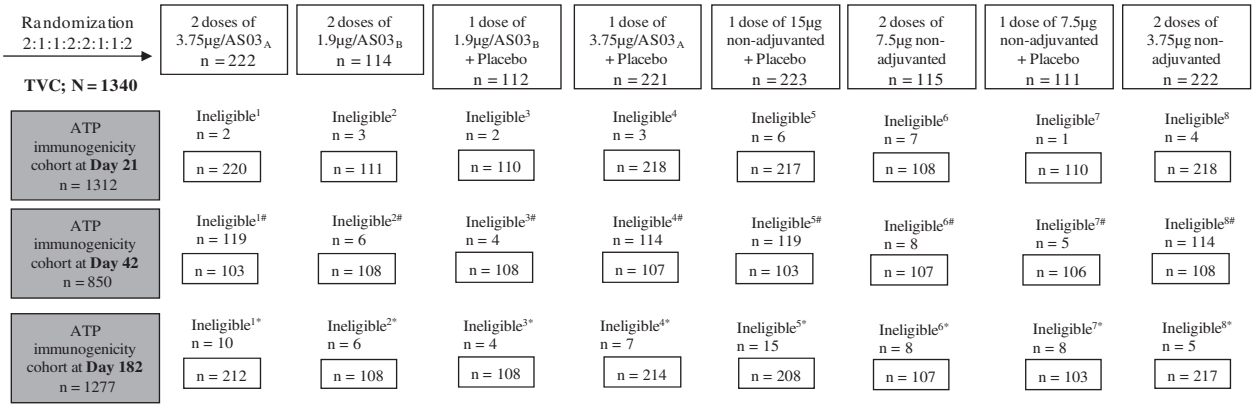
Written informed consent was obtained from all subjects before any study-related procedures were performed. The study was conducted in accordance with the principles of Good Clinical Practice, the Declaration of Helsinki, and relevant local regulations. All study-related documents and procedures were approved by the appropriate ethics committees.

Serological Assessments

Blood samples collected at days 0, 21, 42, and 182 were analyzed at GSK Biologicals' Central Laboratory with a validated in-house hemagglutination-inhibition (HI) assay (cutoff, ≥1:10), using chicken erythrocytes as described elsewhere [7]. The A/California/07/2009 vaccine strain was used as the antigen strain. The assessment of immunogenicity was based on the seroconversion rate (SCR) (percentage of subjects with prevaccination titers <1:10 and postvaccination titers ≥1:40 or with prevaccination titers >1:10 and ≥4-fold increase in postvaccination titers), seroprotection rate (SPR) (percentage of subjects with postvaccination titers ≥1:40), and seroconversion factor (SCF) (ratio of postvaccination to prevaccination geometric mean titers [GMTs]).

Safety and Reactogenicity Assessments

Subjects used diary cards to record the occurrence and intensity of solicited local and general symptoms during each 7-day postvaccination follow-up period. Intensity of solicited symptoms was graded on a standard 4-grade scale (grades 0–3), with



65 subjects aged >60 years received an additional dose of 7.5µg HA vaccine.

Reasons for exclusion from analyses:

1. Non-compliance with blood sampling schedule (1); Essential serological data missing (1)
2. Non-compliance with blood sampling schedule (2); Essential serological data missing (1)
3. Essential serological data missing (2)
4. Non-compliance with blood sampling schedule (2); Essential serological data missing (1)
5. Administration of vaccine unforeseen in the protocol (2); Protocol violation related to inclusion/exclusion criteria (1); Non-compliance with blood sampling schedule (2); Essential serological data missing (1)
6. Administration of vaccine unforeseen in the protocol (1); Non-compliance with blood sampling schedule (3); Essential serological data missing (3)
7. Administration of vaccine unforeseen in the protocol (1)
8. Administration of vaccine unforeseen in the protocol (1); Non-compliance with blood sampling schedule (1); Essential serological data missing (2)

1# Non-compliance with vaccination schedule (1); Non-compliance with blood sampling schedule (7); Essential serological data missing (3); Subjects not planned to be bled at all time points (108)

2# Non-compliance with vaccination schedule (1); Non-compliance with blood sampling schedule (1); Essential serological data missing (4)

3# Essential serological data missing (4)

4# Protocol-forbidden vaccine (1); Randomization failure (1); Non-compliance with blood sampling schedule (2); Essential serological data missing (1); Subjects not planned to be bled at all time points (109)

5# Protocol forbidden vaccine (4); Non-compliance with vaccination schedule (3); Non-compliance with blood sampling schedule (3); Essential serological data missing (3); Subjects not planned to be bled at all time points (106)

6# Protocol forbidden vaccine (1); Non-compliance with vaccination schedule (2); Non-compliance with blood sampling schedule (2); Essential serological data missing (3)

7# Protocol forbidden vaccine (1); Non-compliance with blood sampling schedule (2); Essential serological data missing (2)

8# Protocol forbidden vaccine (2); Non-compliance with vaccination schedule (1); Non-compliance with blood sampling schedule (1); Essential serological data missing (2); Subjects not planned to be bled at all time points (108)

1* Non-compliance with vaccination schedule (1); Non-compliance with blood sampling schedule (1); Essential serological data missing (8)

2* Protocol forbidden vaccine (1); Non-compliance with vaccination schedule (1); Essential serological data missing (4)

3* Non-compliance with blood sampling schedule (1); Essential serological data missing (3)

4* Protocol forbidden vaccine (1); Randomization failure (1); Non-compliance with vaccination schedule (2); Essential serological data missing (3)

5* Protocol forbidden vaccine (4); Protocol violation (Inclusion/Exclusion) (1); Protocol forbidden medication (1); Non-compliance with vaccination schedule (3); Non-compliance with blood sampling schedule (1); Essential serological data missing (5)

6* Protocol forbidden vaccine (1); Non-compliance with vaccination schedule (3); Non-compliance with blood sampling schedule (1); Essential serological data missing (3)

7* Protocol forbidden vaccine (1); Protocol-forbidden medication (3); Non-compliance with blood sampling schedule (4); Essential serological data missing (2)

8* Protocol forbidden vaccine (2); Non-compliance with vaccination schedule (1); Non-compliance with blood sampling schedule (1); Essential serological data missing (1)

Figure 1. Study design plus CONSORT.

grade 1 symptoms being noticeable but without impact on normal activities and grade 3 symptoms being those that prevented normal daily activities (grade 3 redness and swelling, diameter >100 mm; grade 3 fever, oral temperature ≥39°C [≥102.2°F]). The incidence rates of solicited and unsolicited adverse events were calculated with 95% confidence intervals (CIs). Also recorded throughout the study period were unsolicited adverse events occurring during the 21 days after each vaccine dose and 84 days after the first dose and serious adverse events (SAEs) and potential immune-mediated diseases (pIMDs) (adverse events that include both autoimmune diseases and other inflammatory and/or neurologic disorders that may or may not have an autoimmune etiology, an area of interest for regulatory agencies evaluating the safety of new adjuvants). Clinical laboratory parameters were assessed at each visit up to day 42 and at day 182. Causality was assessed by investigators for all reported adverse events, except for solicited local symptoms that were assumed to be related. Urine pregnancy tests were performed for women with childbearing potential before each dose (days 0 and 21), and the results were available before vaccination.

Statistical Analyses

The sample size was calculated taking into consideration the primary objective of evaluating fulfillment of the Center for Biologics Evaluation and Research (CBER) and the CHMP guidance criteria for HI end points after dose 1 of AS03-adjuvanted 3.75- or 1.9-µg HA (H1N1 2009) vaccine (evaluated sequentially) (Table 1) [8, 9]. The evaluable population included 1200 subjects (~95% of 1260 planned; 210 subjects in groups that received either 2 doses of $AS03_A$-adjuvanted or nonadjuvanted 3.75-µg HA H1N1 2009 vaccine or a single dose of 3.75-µg HA/$AS03_A$ or nonadjuvanted 15-µg HA vaccine and 105 subjects in groups that received either 1 or 2 doses of 1.9-µg HA/$AS03_B$ or nonadjuvanted 7.5-µg HA vaccine) and was estimated to provide a power of 99.9% to achieve the primary objectives, assuming 90% and 78% as references for SPR and SCR in subjects aged 18–64 years or >64 years, respectively.

As secondary objectives, significant adjuvant effects were defined according to the lower limit of the 95% CI for the HI antibody GMT ratio between adjuvanted and nonadjuvanted

Table 1. Regulatory Criteria for Evaluation of Immunogenicity of H1N1 2009 Vaccines[a]

Criteria and Age Group	SCR	SPR	SCF
CBER criteria			
18–64 years	Lower limit of 95% CI ≥40%	Lower limit of 95% CI >70%	…
≥64 years	Lower limit of 95% CI ≥30%	Lower limit of 95% CI >60%	…
CHMP criteria			
18–60 years	Point estimate >40%	Point estimate >70%	Point estimate >2.5
>60 years	Point estimate >30%	Point estimate >60%	Point estimate >2.0

[a] CBER, Center for Biologics Evaluation and Research; CHMP, Committee for Medicinal Products for Human Use; CI, confidence interval; SCF, seroconversion factor; SCR, seroconversion rate; SPR, seroprotection rate.

formulations; the criteria for superiority and noninferiority, respectively, were >1.5 and >.67 for this value (Table 2). The analyses of immunogenicity and safety reported here were performed in the according-to-protocol cohort and total-vaccinated cohort (subjects who received ≥1 vaccine dose), respectively.

Table 2. Statistical Criteria for Evaluation of Immunogenicity of H1N1 2009 Vaccines[a]

Comparison and Age Group	Adjusted GMT (95% CI)	Outcome
3.75-μg HA/AS03$_A$ vaccine vs 3.75-μg HA nonadjuvanted vaccine		Superiority established
18–64 years	3.76 (2.86–4.94)	
>64 years	2.78 (2.19–3.52)	
18–60 years	3.87 (2.94–5.11)	
>60 years	2.78 (2.20–3.51)	
3.75-μg HA/AS03$_A$ vaccine vs 7.5-μg HA nonadjuvanted vaccine		Noninferiority established
18–64 years	1.75 (1.34–2.28)	
>64 years	2.80 (2.23–3.52)	
18–60 years	1.86 (1.43–2.42)	
>60 years	2.64 (2.10–3.33)	
3.75-μg HA/AS03$_A$ vaccine vs 15-μg HA nonadjuvanted vaccine		Noninferiority established
18–64 years	2.60 (2.00–3.37)	
>64 years	1.80 (1.43–2.27)	
18–60 years	2.71 (2.09–3.52)	
>60 years	1.81 (1.44–2.27)	
1.9-μg HA/AS03$_B$ vaccine vs 3.75-μg HA nonadjuvanted vaccine		Noninferiority established
18–64 years	2.15 (1.55–2.99)	
>64 years	1.94 (1.45–2.60)	
18–60 years	2.33 (1.64–3.31)	
>60 years	1.85 (1.39–2.45)	
1.9-μg HA/AS03$_B$ vaccine vs 7.5-μg HA nonadjuvanted vaccine		Noninferiority established
18–64 years	0.99 (.72–1.35)	
>64 years	1.92 (1.45–2.54)	
18–60 years	1.12 (.81–1.56)	
>60 years	1.75 (1.34–2.30)	
1.9-μg HA/AS03$_B$ vaccine vs 15-μg HA nonadjuvanted vaccine		Noninferiority established
18–64 years	1.48 (1.08–2.01)	
>64 years	1.23 (.93–1.63)	
18–60 years	1.63 (1.18–2.26)	
>60 years	1.18 (.90–1.54)	

Abbreviation: HA, hemagglutinin.

[a] Results for the according-to-protocol cohort for immunogenicity. The superiority of an adjuvanted formulation was established if the lower limit of the 95% confidence interval (CI) on the geometric mean titer (GMT) ratio at day 21 was >1.5. The noninferiority of an adjuvanted formulation was established if the lower limit of the 95% CI on the GMT ratio at day 21 was >.67.

Table 3. Immune Response in Terms of Hemagglutination-Inhibition Antibodies Against H1N1 2009 Influenza Strain in Subjects Aged 18–64 or >64 Years (CBER Criteria)[a]

Immune Response [CBER Criteria][b]	2 Doses of 3.75 μg HA/AS03$_A$	2 Doses of 1.9 μg HA/AS03$_B$	1.9 μg HA/AS03$_B$ + Placebo	3.75 μg HA/AS03$_A$ + Placebo	15 μg HA + Placebo	2 Doses of 7.5 μg HA	7.5 μg HA + Placebo	2 Doses of 3.75 μg HA
18–64-y Age Group								
Age, mean (range), years	41.4 (18–64)	40.5 (18–64)	41.6 (19–63)	40.3 (19–63)	41.0 (19–64)	41.3 (18–64)	41.4 (20–64)	40.4 (18–64)
Subjects, No.								
Day 21	92	46	47	89	89	46	47	91
Day 42	42	44	45	44	42	45	47	43
Day 182	88	47	46	86	85	45	44	89
SCR, % (95% CI) [LL of 95% CI ≥40%]								
Day 21	91.3 (83.6–96.2)	82.6 (68.6–92.2)	78.7 (64.3–89.3)	88.8 (80.3–94.5)	71.9 (61.4–80.9)	80.4 (66.1–90.6)	78.7 (64.3–89.3)	60.4 (49.6–70.5)
Day 42	90.5 (77.4–97.3)	93.2 (81.3–98.6)	73.3 (58.1–85.4)	88.6 (75.4–96.2)	**54.8 (38.7–70.2)**	88.9 (75.9–96.3)	76.6 (62.0–87.7)	65.1 (49.1–79.0)
Day 182	89.8 (81.5–95.2)	80.9 (66.7–90.9)	56.5 (41.1–71.1)	68.6 (57.7–78.2)	58.8 (47.6–69.4)	68.9 (53.4–81.8)	72.7 (57.2–85.0)	51.7 (40.8–62.4)
SPR,% (95% CI) [LL of 95% CI ≥70%]								
Day 0	20.7 (12.9–30.4)	23.9 (12.6–38.8)	17.0 (7.6–30.8)	19.1 (11.5–28.8)	22.5 (14.3–32.6)	17.4 (7.8–31.4)	14.9 (6.2–28.3)	15.4 (8.7–24.5)
Day 21	98.9 (94.1–100)	97.8 (88.5–99.9)	87.2 (74.3–95.2)	95.5 (88.9–98.8)	88.8 (80.3–94.5)	89.1 (76.4–96.4)	95.7 (85.5–99.5)	74.7 (64.5–83.3)
Day 42	100 (91.6–100)	100 (92.0–100)	84.4 (70.5–93.5)	90.9 (78.3–97.5)	**83.3 (68.6–93.0)**	97.8 (88.2–99.9)	93.6 (82.5–98.7)	**79.1 (64.0–90.0)**
Day 182	96.6 (90.4–99.3)	89.4 (76.9–96.5)	**73.9 (58.9–85.5)**	83.7 (74.2–90.8)	**76.5 (66.0–85.0)**	**82.2 (67.9–92.0)**	**84.1 (69.9–93.4)**	**68.5 (57.8–78.0)**
>64-y Age Group								
Age, mean (range), years	70.7 (65–85)	72.1 (65–85)	72.1 (65–85)	71.4 (65–87)	71.2 (65–85)	71.6 (65–87)	71.6 (65–90)	71.6 (65–89)
Subjects, No.								
Day 21	128	65	63	129	128	62	63	127
Day 42	61	64	63	63	61	62	59	65
Day 182	124	61	62	128	123	62	59	128
SCR, % (95% CI) [LL of 95% CI ≥30%]								
Day 21	77.3 (69.1–84.3)	63.1 (50.2–74.7)	55.6 (42.5–68.1)	79.1 (71.0–85.7)	49.2 (40.3–58.2)	**35.5 (23.7–48.7)**	**36.5 (24.7–49.6)**	**36.2 (27.9–45.2)**
Day 42	85.2 (73.8–93.0)	78.1 (66.0–87.5)	55.6 (42.5–68.1)	76.2 (63.8–86.0)	59.0 (45.7–71.4)	45.2 (32.5–58.3)	**30.5 (19.2–43.9)**	50.8 (38.1–63.4)
Day 182	68.5 (59.6–76.6)	52.5 (39.3–65.4)	**25.8 (15.5–38.5)**	41.4 (32.8–50.4)	**37.4 (28.8–46.6)**	**37.1 (25.2–50.3)**	**28.8 (17.8–42.1)**	**30.5 (22.6–39.2)**
SPR, % (95% CI) [LL of 95% CI ≥60%]								
Day 0	11.7 (6.7–18.6)	12.3 (5.5–22.8)	19.0 (10.2–30.9)	14.7 (9.1–22.0)	17.2 (11.1–24.9)	9.7 (3.6–19.9)	17.5 (9.1–29.1)	13.4 (8.0–20.6)
Day 21	89.8 (83.3–94.5)	76.9 (64.8–86.5)	73.0 (60.3–83.4)	92.2 (86.2–96.2)	**66.4 (57.5–74.5)**	**48.4 (35.5–61.4)**	**55.6 (42.5–68.1)**	**52.8 (43.7–61.7)**
Day 42	96.7 (88.7–99.6)	85.9 (75.0–93.4)	74.6 (62.1–84.7)	88.9 (78.4–95.4)	**70.5 (57.4–81.5)**	**59.7 (46.4–71.9)**	**54.2 (40.8–67.3)**	**61.5 (48.6–73.3)**
Day 182	79.8 (71.7–86.5)	**67.2 (54.0–78.7)**	**50.0 (37.0–63.0)**	**64.8 (55.9–73.1)**	**55.3 (46.1–64.3)**	**46.8 (34.0–59.9)**	**49.2 (35.9–62.5)**	**50.8 (41.8–59.7)**

Bold values did not meet CBER immunogenicity guidance criteria.

Abbreviations: CI, confidence interval; LL, lower limit SCR, seroconversion rate; SPR, seroprotection rate.

[a] Data from according-to-protocol cohort for immunogenicity.

[b] Center for Biologics Evaluation and Research (CBER) criteria are indicated in brackets.

Table 4. Immune Response in Terms of Hemagglutination-Inhibition Antibodies Against H1N1 2009 Influenza Strain in Subjects Aged 18–60 or >60 Years (CHMP Criteria)[a]

Immune Response [CHMP Criteria][b]	2 Doses of 3.75 μg HA/AS03$_A$	2 Doses of 1.9 μg HA/AS03$_B$	1.9 μg HA/AS03$_B$ + Placebo	3.75 μg HA/AS03$_A$ + Placebo	15 μg HA + Placebo	2 Doses of 7.5 μg HA	7.5 μg HA + Placebo	2 Doses of 3.75 μg HA
18-60-y Age Group								
Age, mean (range), years	38.3 (18–60)	37.9(18–60)	39.1 (19–60)	38.8 (19–60)	38.7 (19–60)	39.9 (18–60)	39.4 (20-60)	38.0 (18–60)
Subjects, No.								
Day 21	80	41	42	83	80	44	43	82
Day 42	39	39	40	40	39	43	43	39
Day 182	76	42	41	80	76	43	40	80
SCR, % (95% CI) [>40%]								
Day 21	92.5 (84.4–97.2)	82.9 (67.9–92.8)	83.3 (68.6–93.0)	90.4 (81.9–95.7)	72.5 (61.4–81.9)	79.5 (64.7–90.2)	76.75 (61.4–88.2)	59.85 (48.3–70.4)
Day 42	92.3 (79.1–98.45)	94.9 (82.7–99.4)	77.55 (61.5–89.2)	92.5 (79.6–98.4)	53.8 (37.2–69.9)	88.4 (74.9–96.1)	74.4 (58.8–86.5)	64.1 (47.2–78.8)
Day 182	93.4 (85.3–97.8)	85.7 (71.5–94.6)	63.4 (46.9–77.9)	71.3 (60.0–80.8)	57.9 (46.0–69.1)	67.4 (51.5–80.9)	70.0 (53.5–83.4)	56.3 (44.7–67.3)
SPR, % (95% CI) [>70%]								
Day 0	22.5 (13.9–33.2)	24.4 (12.4–40.3)	16.7 (7.0–31.4)	20.5 (12.4–30.8)	23.8 (14.9–34.6)	15.9 (6.6–30.1)	14.0 (5.3–27.9)	15.9 (8.7–25.6)
Day 21	98.8 (93.2–100)	97.6 (87.1–99.9)	90.5 (77.4–97.3)	97.6 (91.6–99.7)	88.8 (79.7–94.7)	88.6 (75.4–96.2)	95.3 (84.2–99.4)	74.4 (63.6–83.4)
Day 42	100 (91.0–100)	100 (91.0–100)	87.5 (73.2–95.8)	95.0 (83.1–99.4)	82.1 (66.5–92.5)	97.7 (87.7–99.9)	93.0 (80.9–98.5)	76.9 (60.7–88.9)
Day 182	98.7 (92.9–100)	92.9 (80.5–98.5)	80.5 (65.1–91.2)	87.5 (78.2–93.8)	76.3 (65.2–85.3)	81.4 (66.6–91.6)	82.5 (67.2–92.7)	71.3 (60.0–80.8)
SCF (95% CI) [>2.5]								
Day 21	29.9 (22.4–40.0)	20.1 (13.0–31.2)	17.0 (11.4–25.2)	30.2 (22.4–40.7)	10.0 (7.3–13.6)	15.9 (10.4–24.2)	21.1 (12.9–34.5)	9.1 (6.6–12.7)
Day 42	38.3 (25.6–57.2)	32.9 (21.2–51.0)	12.8 (8.7–18.8)	22.4 (15.0–33.6)	6.6 (4.1–10.6)	18.7 (12.7–27.4)	18.5 (11.5–29.7)	8.9 (5.7–13.8)
Day 182	17.2 (12.8–23.3)	12.7 (8.5–19.1)	6.8 (4.7–9.8)	11.1 (8.2–15.1)	6.1 (4.4–8.5)	9.0 (6.1–13.4)	12.3 (7.5–20.4)	6.4 (4.8–8.5)
>60-y Age Group								
Age, mean (range), years	70.0 (61–85)	71.4 (61–85)	71.4 (61–85)	71.0 (61–87)	70.6 (61–85)	71.3 (63–87)	71.1 (61–90)	71.0 (61–89)
Subjects, No.								
Day 21	140	70	68	135	137	64	67	136
Day 42	64	69	68	67	64	64	63	69
Day 182	136	66	67	134	132	64	63	147
SCR, % (95% CI) [>30%]								
Day 21	77.9 (70.1–84.4)	64.3 (51.9–75.4)	54.4 (41.9–66.5)	78.5 (70.6–85.1)	50.4 (41.7–59.0)	37.5 (25.7–50.5)	40.3 (28.5–53.0)	38.2 (30.0–47.0)
Day 42	84.4 (73.1–92.2)	78.3 (66.7–87.3)	54.4 (41.9–66.5)	74.6 (62.5–84.5)	59.4 (46.4–71.5)	46.9 (34.3–59.8)	34.9 (23.3–48.0)	52.2 (39.8–64.4)
Day 182	68.4 (59.9–76.1)	51.5 (38.9–64.0)	23.9 (14.3–35.9)	41.0 (32.6–49.9)	39.4 (31.0–48.3)	39.1(27.1–52.1)	33.3 (22.0–46.3)	**29.2 (21.7–37.6)**
SPR, % (95% CI) [>60%]								
Day 0	11.4 (6.7–17.9)	12.9 (6.1–23.0)	19.1 (10.6–30.5)	14.1 (8.7–21.1)	16.8 (11.0–24.1)	10.9 (4.5–21.2)	17.9 (9.6–29.2)	13.2 (8.0–20.1)
Day 21	90.7 (84.6–95.0)	78.6 (67.1–87.5)	72.1 (59.9–82.3)	91.1 (85.0–95.3)	67.9 (59.4–75.6)	**50.0 (37.2–62.8)**	**58.2 (45.5–70.2)**	**54.4 (45.7–63.0)**
Day 42	96.9 (89.2–99.6)	87.0 (76.7–93.9)	73.5 (61.4–83.5)	86.6 (76.0–93.7)	71.9 (59.2–82.4)	60.9 (47.9–72.9)	57.1 (44.0–69.5)	63.8 (51.3–75.0)
Day 182	80.1 (72.4–86.5)	66.7 (54.0–77.8)	47.8 (35.4–60.3)	63.4 (54.7–71.6)	**56.8 (47.9–65.4)**	**48.4 (35.8–61.3)**	**52.4 (39.4–65.1)**	**50.4 (41.7–59.0)**

Table 4 continued.

Immune Response [CHMP Criteria][b]	2 Doses of 3.75 µg HA/AS03$_A$	2 Doses of 1.9 µg HA/AS03$_B$	1.9 µg HA/AS03$_B$ + Placebo	3.75 µg HA/AS03$_A$ + Placebo	15 µg HA + Placebo	2 Doses of 7.5 µg HA	7.5 µg HA + Placebo	2 Doses of 3.75 µg HA
SCF (95% CI) [>2.0]								
Day 21	11.8 (9.6–14.4)	8.3 (6.0–11.3)	6.7 (5.0–8.9)	9.9 (8.1–12.1)	6.1 (4.9–7.6)	4.8 (3.3–6.8)	3.8 (2.9–5.1)	3.8 (3.1–4.8)
Day 42	21.1 (14.8–29.9)	14.2 (10.2–19.7)	5.8 (4.4–7.6)	8.1 (6.2–10.4)	6.0 (4.3–8.5)	5.6 (4.0–7.7)	3.2 (2.4–4.3)	5.1 (3.8–6.8)
Day 182	8.4 (6.9–10.3)	5.8 (4.3–7.7)	2.8 (2.1–3.7)	4.2 (3.5–5.0)	3.5 (2.8–4.4)	3.5 (2.5–4.9)	2.9 (2.3–3.8)	2.8 (2.4–3.4)

Bold values did not meet CHMP immunogenicity guidance criteria.

Abbreviations: CI, confidence interval; LL, lower limit SCF, seroconversion factor; SCR, seroconversion rate; SPR, seroprotection rate.

[a] Data from according-to-protocol cohort for immunogenicity.

[b] Committee for Medicinal Products for Human Use (CHMP) criteria are indicated in brackets.

RESULTS

Demography

The study was conducted between October 2009 and May 2010. A total of 1836 subjects were enrolled, of whom 1340 were vaccinated, 1309 completed the study up to day 182, and ATP cohort for immunogenicity was 1277 at day 182 (Figure 1). The mean age of vaccinated subjects at dose 1 was 58.7 years (range, 18–90 years; refer to Tables 3 and 4 for mean ages by age strata); 60.9% of subjects were female. The majority of subjects (88.9%) were of white/European heritage; the principal minority ethnic group was African American (5.6%). The overall demographic profiles of the study groups were comparable.

Immune Response

The group-wise immunogenicity data by age strata at all time points are presented in Tables 3 and 4. Prevaccination baseline SPRs in the various adjuvanted and nonadjuvanted treatment groups varied between 15.4% and 22.5% in the 18–64-year age stratum (14.9%–23.8% in the 18−60-year stratum) and between 13.2% and 17.2% in the >64-year age stratum (12.7%–16.8% in the >60-year stratum). The first dose of 3.75-µg HA/AS03$_A$ and 1.9-µg HA/AS03$_B$ induced immune responses in both age strata that substantially exceeded the CBER guidance criteria for pandemic influenza vaccines (Table 1). The CHMP guidance criteria were exceeded in the 18−60-year and >60-year age strata (Table 1). The nonadjuvanted 15-µg HA formulations also induced immune responses that met the CBER and CHMP immunogenicity criteria in both age strata (Table 1). In contrast, the nonadjuvanted 7.5- and 3.75-µg HA formulations induced immune responses that met the CBER criteria only in the 18−64-year age stratum. The CHMP guidance criteria were met only in subjects aged 18–60 years who received the nonadjuvanted 15- and 7.5-µg HA formulations.

In younger subjects (18−60 years old), HI antibody GMTs were higher at day 21 for those subjects who were seropositive at baseline in all groups (289.2 to 484.7 vs 183.7 to 318.4 for AS03-adjuvanted groups; 161.3 to 230 vs 67.9 to 169 for nonadjuvanted groups); however, the CIs were all overlapping, except for the 3.75-µg nonadjuvanted group. In older subjects (>60 years), HI antibody GMTs were higher at day 21 for those subjects seropositive at baseline in all groups (111.5 to 176 vs 63.8 to 87.3 for adjuvanted groups; 68.1 to 126.3 vs 26.7 to 36.5 for nonadjuvanted groups); the CIs were all nonoverlapping, except for the 1.9-µg HA AS03$_B$-adjuvanted group. In addition, the effect of adjuvant was evident in terms of HI antibody GMT ratios between the 3.75-µg HA AS03$_A$-adjuvanted and nonadjuvanted groups (by baseline serostatus), which varied between 3.0 (baseline seropositive) and 4.7 (baseline seronegative) in subjects aged 18−60 years and between 2.6 (baseline seropositive) and 3.3 (baseline seronegative) in subjects aged >60 years. After dose 2, all treatment groups except the group receiving 2 doses of nonadjuvanted

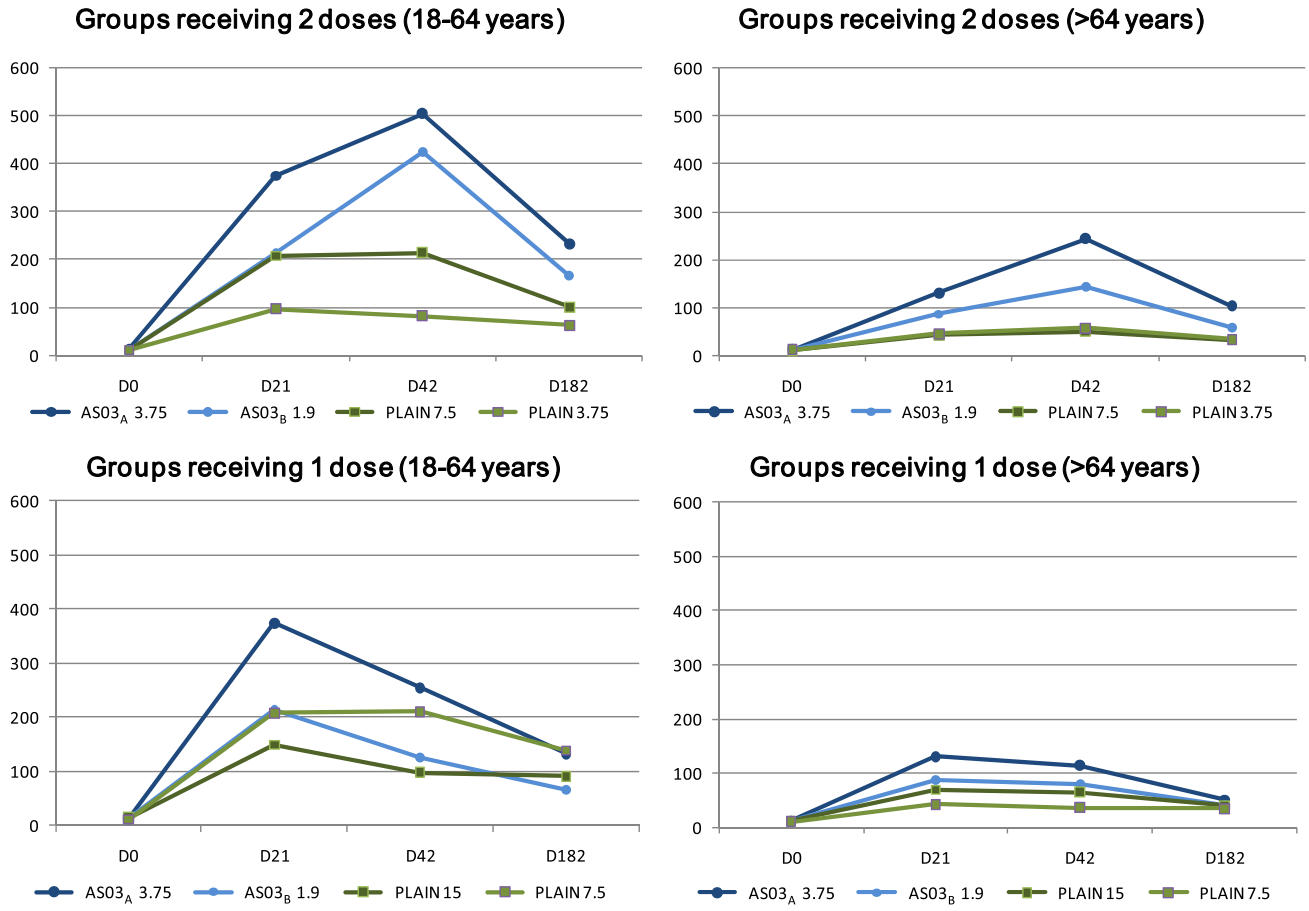


Figure 2. Hemagglutination inhibition antibody geometric mean titers at different time points (according-to-protocol cohort for immunogenicity).

3.75-μg HA formulation met the CBER immunogenicity criteria in the 18−64-year age stratum; in the >64-year age stratum, only the adjuvanted formulations induced immune responses that met the CBER criteria (Table 1). For the CHMP criteria, in the 18−60-year age stratum, all treatment groups, except the nonadjuvanted 15-μg HA formulation when given once, met the immunogenicity cutoffs; in the >60-year age stratum, only the adjuvanted formulations met the CHMP criteria (Table 1).

After an additional dose of nonadjuvanted 7.5-μg HA formulation in 65 subjects aged >60 years who were scheduled to receive only a single dose, the CHMP immunogenicity criteria were met (day 63 SCR, 44.1% [95% CI, 31.2%–57.6%]; SPR, 64.4% [95% CI, 50.9%–76.4%]; SCF, 4.2 [95% CI, 3.2–5.6]); however, the CBER immunogenicity criteria for SPR were not met.

At month 6, only subjects who received either 2 doses of the adjuvanted 3.75- or 1.9-μg HA formulations or 1 dose of the adjuvanted 3.75-μg HA formulation met the CBER and CHMP criteria in the 18−64- and 18−60-year age strata, respectively. In the >64- and >60-year age strata, subjects who received 2 doses of the adjuvanted 3.75-μg HA formulation met the 2 criteria, respectively, and subjects >60 years old also met CHMP criteria after the receipt of a single dose of the adjuvanted 3.75-μg HA vaccine (Tables 3 and 4). None of the nonadjuvanted treatment groups met the CBER or CHMP criteria at month 6. Subjects aged >60 years who received the additional dose of nonadjuvanted 7.5-μg HA formulation still met the CHMP criteria at 6 months after the first vaccine dose (day 182 SCR, 32.2% [95% CI, 21.1%–45.1%]; SPR, 50.8% [95% CI, 38.1%–63.4%]; SCF, 2.9 [95% CI, 2.2–3.7]). Of the 2 adjuvanted formulations, the 3.75-μg HA/AS03$_A$ formulation induced a stronger immune response than the 1.9-μg HA/AS03$_B$ formulation in both age strata. The corresponding HI antibody GMTs for subjects aged 18−64 or >64 years are presented in Figure 2.

The nonadjuvanted formulations (7.5- and 15-μg HA) induced lower immune responses than the 2 adjuvanted formulations. Among the nonadjuvanted formulations, the 7.5- and 15-μg HA formulations induced similar immune response in subjects aged 18–64 and those aged 18−60 years, whereas the 15-μg HA formulation induced the strongest immune response in subjects aged >64 or >60 years.

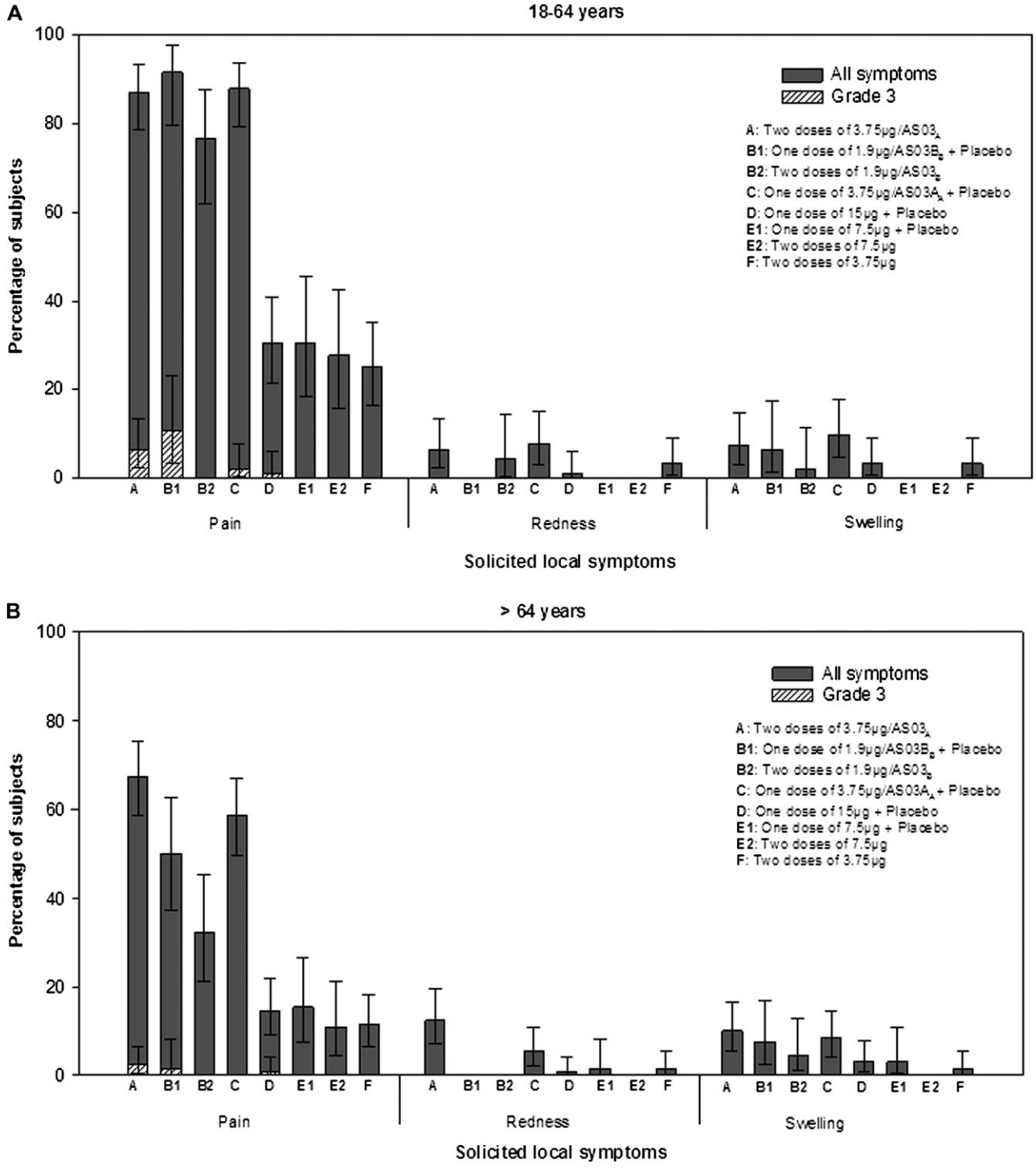


Figure 3. *A, B,* Percentages of subjects reporting solicited local adverse events during 7-day postvaccination follow-up period after dose 1, stratified by age (total vaccinated cohort).

The effect of added adjuvant was established at day 21 according to predefined criteria. The adjuvanted 3.75-µg HA formulation was demonstrated to be superior to the nonadjuvanted 3.75- and 15-µg HA formulations in subjects aged 18−64 years and the nonadjuvanted 7.5-µg HA formulation in subjects aged >64 years and noninferior to the other formulations. Similarly, the adjuvanted 1.9-µg HA formulation was demonstrated to be superior to the nonadjuvanted 3.75-µg HA formulation in subjects aged 18−64 years and noninferior to the other formulations (Table 2).

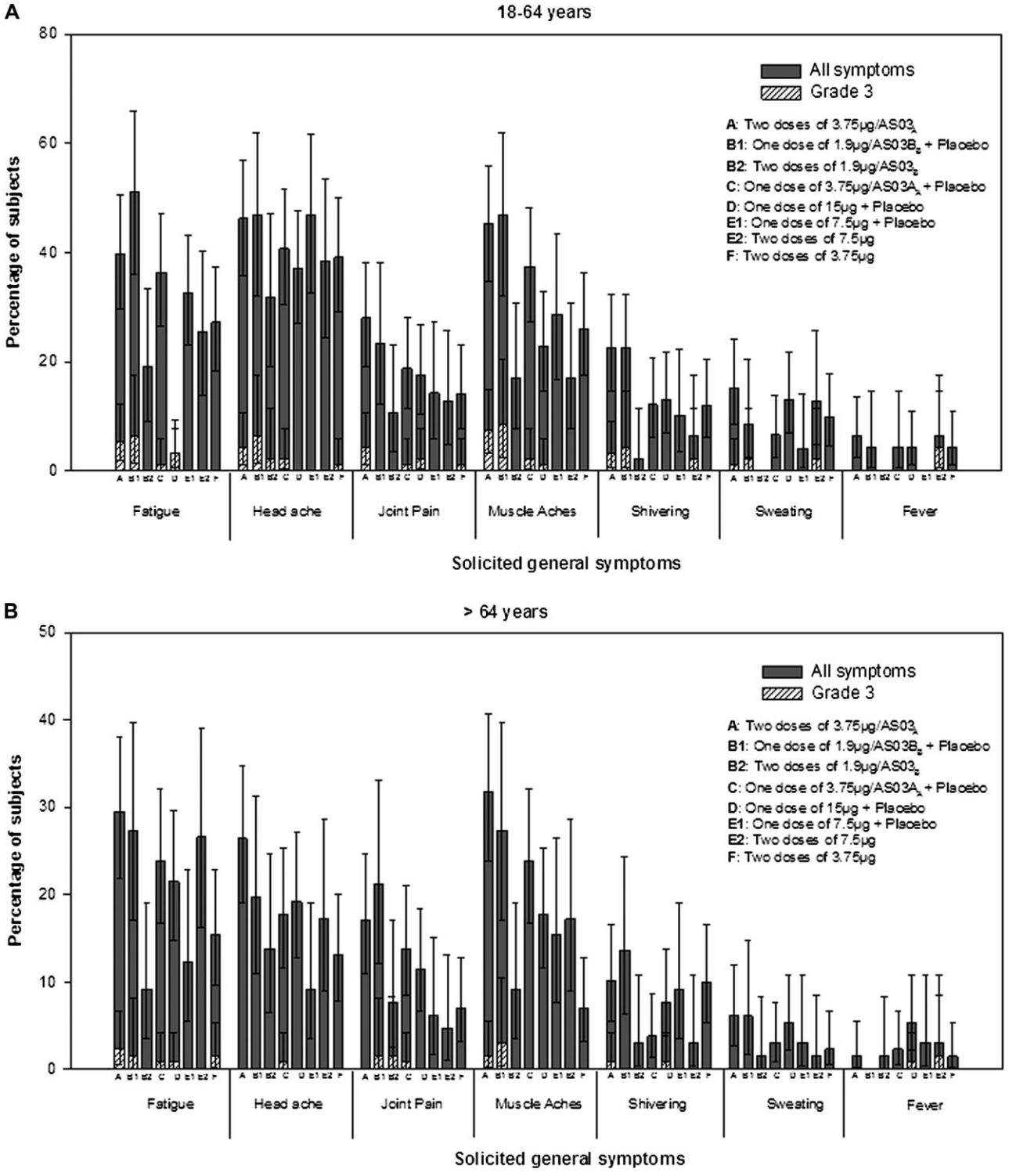


Figure 4. *A, B,* Percentages of subjects reporting solicited general adverse events during 7-day postvaccination follow-up period after dose 1, stratified by age (total vaccinated cohort).

Safety and Reactogenicity

The frequency of reporting of all solicited local and general symptoms in subjects aged 18–64 or >64 years is presented in Figures 3 and 4. Injection site pain was the most frequently recorded solicited local adverse event in both age strata (18–64 years, 25.0%–91.5%; >64 years, 10.9%–67.4%), regardless of the formulation received, and was reported more often by subjects who received the AS03-adjuvanted formulations. Grade

3 injection site pain was reported in <11.0% of subjects aged 18–64 years and <3.0% of subjects aged >64 years who received the AS03-adjuvanted formulations. The overall incidences of solicited general adverse events were comparable across the AS03-adjuvanted and nonadjuvanted formulations, with the exception of muscle ache, which was reported more frequently by subjects who received the AS03-adjuvanted formulations (17.0%–46.8%) than by those who received the nonadjuvanted formulations (17.0%–28.6%). Headache, fatigue, and muscle ache were the most frequently reported solicited general adverse events (18–64 years, 31.9%–46.9%, 19.1%–51.1%, and 17.0%–46.8%, respectively; >64 years, 9.2%–26.4%, 9.2$–29.5%, and 9.2%–31.8%, respectively). Grade 3 headache, fatigue, and muscle ache were reported in <7.0% and <4.0% of subjects across adjuvanted and nonadjuvanted groups, respectively. The overall reporting of both local and general adverse events was more frequent in the 18–64-year age group. In subjects aged >60 years (n = 65) who received the additional dose of nonadjuvanted 7.5-μg HA formulation, pain at injection site, headache, fatigue, and muscle aches were the most frequently reported solicited adverse events (9.4%, 9.5%, 9.5%, and 6.3% of subjects, respectively).

Reports of unsolicited adverse events up to 84 days after the first vaccine dose were similar in subjects who received the AS03-adjuvanted and nonadjuvanted formulations. The most frequently reported unsolicited adverse event after vaccination with the AS03-adjuvanted formulations was oropharyngeal pain (<15.0% of subjects aged 18–64 years) and cough and nasopharyngitis (<7.0% of subjects aged >64 years). After vaccination with nonadjuvanted vaccines, it was headache and oropharyngeal pain (<9.0%) in both age strata. Unsolicited adverse events of grade 3 severity were reported at similar rates (<11.0% of subjects) across all study groups. Thirteen subjects aged >60 years who received the additional dose of nonadjuvanted 7.5-μg HA formulation reported ≥1 unsolicited adverse event (all events reported by 1 subject each).

Fifty-nine SAEs were reported for 39 subjects up to day 182. One SAE (thrombocytopenia; 2 doses of 3.75-μg HA/AS03$_A$) was assessed by the investigator as causally related to vaccination. Two SAEs were fatal; 1 subject died of an acute myocardial infarction and another subject died of pancreatic carcinoma with metastases in the liver (both received 2 doses of 3.75-μg HA/AS03$_A$). Three potential immune-mediated diseases were reported for 3 subjects receiving the AS03-adjuvanted vaccines (thrombocytopenia secondary to sulfa allergy, thrombocytopenia in a subject with a history of hematuria noted above as an SAE, and autoimmune thyroiditis after biopsy for a woman with long-standing thyroid disease). No major changes in clinical laboratory parameters were recorded from the baseline values. Six pregnancies were identified by day 182: 4 were ongoing, 1 subject underwent elective abortion, and 1 experienced a spontaneous abortion.

DISCUSSION

Consistent with data from previous studies with adjuvanted and nonadjuvanted H1N1 2009 influenza vaccines in adults including elderly adults [10–12], a single dose of 3.75-μg HA/AS03$_A$ or 1.9-μg HA/AS03$_B$ met CBER and CHMP guidance criteria for pandemic influenza vaccines. Vaccine containing 3.75-μg HA/AS03$_A$ was more immunogenic than nonadjuvanted 15-μg HA. The SPR induced by the nonadjuvanted 15-μg HA formulation in this study (age 18–64 years, 88.8%; >64 years, 66.4%) was at the lower end of the spectrum of observations in other studies using nonadjuvanted 15-μg HA formulations (SPR, 85%−98%) [11–13]. A similar observation was made for the nonadjuvanted 7.5-μg HA formulation, wherein relatively low SPRs (89.1% and 48.4%, respectively) were observed compared with those reported elsewhere (89.5%–95% and 80.3%–94% in younger and elderly adults, respectively) [12, 13]. However, these comparisons require careful consideration because the samples were tested at different laboratories and the estimated HA antigen contents for the nonadjuvanted 15- and 7.5-μg HA formulations in the present study were lower than planned (11- and 5.6-μg HA, respectively [SRID results]). Nonetheless, immune responses were vigorous and there was little dose-response in younger subjects, as evident from the comparable immune responses to the actual doses of nonadjuvanted 11- and 5.6-μg HA formulations (intended dose, 15 and 7.5 μg), which were significantly higher than with the nonadjuvanted 3.75-μg HA formulation.

Although no difference was observed in the immune response induced by the 2 adjuvanted formulations in the 18–64-year age group, the >64-year age group showed a stronger response to the 3.75-μg HA/AS03$_A$ formulation. For the nonadjuvanted formulations, subjects aged >64 years responded with higher HI titers to a higher antigen dose (15-μg HA) than subjects aged 18–64 years who responded at least as well to the nonadjuvanted 7.5-μg formulation. The clinical significance of this finding is unknown but suggests that younger adults may be less sensitive to antigen content. Prevaccination serostatus seemed to influence immune response against the vaccine strain in both age groups, irrespective of the vaccine formulation received, and seemed more pronounced in those aged >60 years (day 21 HI antibody GMTs were higher in subjects seropositive at baseline).

Data on antibody persistence in adults and elderly adults after H1N1 2009 vaccination are limited. In this study, 6 months after dose 1, only subjects who received either 2 doses of the AS03-adjuvanted 3.75- or 1.9-μg HA formulations or 1 dose of the AS03-adjuvanted 3.75-μg HA formulation met the CBER (and CHMP criteria) in the 18−64-year (18−60-year) age stratum. In the >64-year (>60-year) stratum, only subjects who received 2 doses of the adjuvanted 3.75-μg HA formulation continued to meet the 2 sets of criteria.

The adjuvanted and nonadjuvanted formulations had a clinically acceptable safety profile in both age strata. As observed in others studies of AS03-adjuvanted H5N1 [4–6] and H1N1 2009 vaccines [13], local reactions (primarily pain) were reported more frequently after vaccination with the AS03-adjuvanted formulations than with the nonadjuvanted formulations. The only vaccine-related SAE (also a potential immune-mediated disease) was reported in a subject who received 2 doses of 3.75-μg HA/AS03$_A$. No difference in the reporting of adverse events was observed between the 3.75- and 1.9-μg formulations of the adjuvanted vaccines.

The occurrence of substantial baseline SPRs (≤23.9%) before vaccination is consistent with data published elsewhere (United Kingdom, 4%–12%; Australia, 30%) [10, 11]. Asymptomatic infections with the H1N1 2009 pandemic influenza strain in circulation in the United States at the time of this study may have contributed to the high baseline SPR observed in the younger subjects.

In conclusion, data from this study suggest that a single dose of the AS03-adjuvanted H1N1 2009 pandemic influenza vaccine with a low HA antigen content (3.75 μg) is highly immunogenic and was well tolerated. The humoral immune response induced after vaccination persisted for ≥6 months after the first vaccine dose. Hence, it could provide a suitable option for immunizing adults including elderly adults. The nonadjuvanted formulation with a higher HA antigen content (15 μg) was also found to be sufficiently immunogenic with a clinically acceptable safety profile, although the immune response was lower than that elicited by the AS03-adjuvanted formulations at all time points.

Notes

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Potential conflicts of interest. M. F., G. R., M. D., E. S., and M. B. are principle investigators in studies funded by GlaxoSmithKline. All investigators received compensation for study involvement and travel related to this study. M. F. is principal investigator at the research site owned by his wife. L. F., O. G., P. L., M. M., and D. V. are employees of GlaxoSmithKline and own stock in the company.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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