MAJOR ARTICLE

Responses to A(H1N1)pdm09 Influenza Vaccines in Participants Previously Vaccinated With Seasonal Influenza Vaccine: A Randomized, Observer-Blind, Controlled Study

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Background. Prior receipt of a trivalent seasonal influenza vaccine (TIV) can affect hemagglutination inhibition (HI) antibody responses to pandemic influenza vaccines. We investigated the effect of TIV priming on humoral responses to AS03-adjuvanted and nonadjuvanted A(H1N1)pdm09 vaccines, the role of AS03 on cell-mediated immune (CMI) responses, and vaccine safety.

Methods. Healthy adults (aged 19–40 years) were randomized 1:1:1:1 to receive TIV or saline followed 4 months later by 2 doses, 3 weeks apart, of adjuvanted or nonadjuvanted A(H1N1)pdm09 vaccine and followed up to study end (day 507). Pre- and postvaccination responses of HI and neutralizing antibody, $CD4^+$/$CD8^+$ T cells, memory B cells, and plasmablasts were assessed.

Results. Ninety-nine of the 133 participants enrolled completed the study. No vaccine-related serious adverse events were recorded. In TIV-primed participants, A(H1N1)pdm09-specific antibody and $CD4^+$ T-cell and memory B-cell responses to the pandemic vaccine tended to be diminished. Vaccine adjuvantation led to increased responses of vaccine-homologous and -heterologous HI and neutralizing antibodies and $CD4^+$ T cells, homologous memory B cells, and plasmablasts.

Conclusions. In healthy adults, prior TIV administration decreased humoral and CMI responses to A(H1N1)pdm09 vaccine. Adjuvantation of A(H1N1)pdm09 antigen helped to overcome immune interference between the influenza vaccines. No safety concerns were observed.

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Keywords. A(H1N1)pdm09 vaccine; AS03; TIV; pandemic influenza; immune interference; HI antibody; neutralizing antibody; $CD4^+$ T cells; B cells; plasmablasts.

From the recognized onset of an H1N1 pandemic in 2009 until its end in August 2010, the swine-origin A/California/7/2009 [A(H1N1)pdm09] virus caused more than 600 000 laboratory-confirmed infections and 18 449 deaths [1, 2], though estimates suggest a death toll of 15 times that amount [3]. In response, a number of A(H1N1)pdm09 vaccines were developed [4–6], several of which were formulated with an adjuvant to enhance immunogenicity and reduce the antigen dose [7–9].

Influenza A(H1N1)pdm09 vaccine clinical studies have shown that the prevaccination serostatus of the studied population is an important determinant for the vaccine's immunogenicity [5, 8–19]. Specifically, results from a number of trials have suggested that prior receipt of a trivalent seasonal influenza vaccine (TIV) or another nonadjuvanted influenza vaccine can affect the hemagglutination inhibition (HI) antibody responses to adjuvanted [8, 10–14] and nonadjuvanted [5, 9, 15–19] A(H1N1)pdm09 or H5N1 [20, 21] vaccines. Several

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authors [16, 22] have suggested that this effect of immune interference is akin to the original antigenic sin mechanism [23], in which sequential exposure to closely related viruses can lead to diminished antibody responses to the novel antigens in the strain of the last exposure. However, the relevance of this concept for sequential influenza vaccinations is subject to debate [24, 25].

In mice, it has been shown that such immune interference effects may be circumvented by adjuvantation of the priming vaccine [26]. This is in agreement with recent clinical studies that show that adjuvants can enhance $CD4^+$ T-cell responses [9, 14, 27]. Increased $CD4^+$ T-cell responses, in turn, could promote B-cell adaptation to antigens that are less related to the specificities in the memory B-cell pool, such as those present in a pandemic influenza vaccine [28, 29]. Thus, $CD4^+$ T-cell responses could play a role in shaping an immune response that is better prepared to respond to the subsequent vaccination. Indeed, clinical studies have shown that Adjuvant System 03 (AS03) [30] enhanced $CD4^+$ T-cell and humoral responses to A(H1N1)pdm09 [9, 14] and H5N1 [27] antigens.

Our aim in this study was to investigate the effect of priming with TIV on subsequent HI and neutralizing antibody (NAb) responses to adjuvanted and nonadjuvanted A(H1N1)pdm09 vaccines. We also evaluated the role of AS03 on the frequency and/or phenotypes of cell-mediated immune (CMI) responses in terms of T cells, memory B cells, and plasmablasts, as well as vaccine safety. We selected a study population (aged 19–40 years) for whom potential immunosenescence effects would not be expected.

METHODS

Study Design

This phase 1/2 randomized observer-blind study (clinicaltrials.gov; NCT01059617) was conducted from February 2010 through January 2011 at 3 study centers in the United States, after approval by an independent local ethics committee. The study was undertaken in accordance with the Helsinki Declaration and good clinical practices. All participants provided written consent before entering the study.

Using an Internet-based algorithm, eligible participants were randomized 1:1:1:1 to 4 parallel groups to receive either TIV followed by 2 doses of adjuvanted (group A) or nonadjuvanted (group B) A(H1N1)pdm09 vaccine, or placebo (saline) followed by 2 doses of adjuvanted (group C) or nonadjuvanted (group D) A(H1N1)pdm09 vaccine (Figure 1). Vaccine doses were administered on day 0 (TIV/placebo; dose 1), followed 4 months later by 2 doses of A(H1N1)pdm09 vaccine (doses 2 and 3) given at 3-week intervals (days 122 and 143). Participants were followed for 364 days after dose 3 (day 507).

Blood samples for immunogenicity and safety evaluations were taken before vaccination (days 0, 122, and 143); 1, 2, and 3 weeks post dose 1 (days 7, 14, and 21); 3, 7, and 14 days post dose 2 (days 125, 129, and 136); and 1, 2, 3, and 23 weeks post dose 3 (days 150, 157, 164, and 304, respectively).

Study Objectives

The primary objective was to describe the homologous and heterologous HI antibody responses following adjuvanted or nonadjuvanted A(H1N1)pdm09 vaccination of individuals vaccinated previously with TIV or placebo. Secondary objectives were to describe the homologous and heterologous NAb and CMI responses to the pandemic vaccine virus, as well as vaccine safety across the study period.

Study Participants

Healthy participants (male and female) aged 19–40 years were enrolled. Exclusion criteria were as follows: previous administration of any influenza vaccine including any A/California/7/2009 (H1N1)v-like virus vaccine, administration of any vaccine within 30 days before first vaccination, use of investigational or nonregistered products, confirmed or suspected immunosuppression, receipt of immunoglobulins or blood products within 9 months preceding the study, and known or suspected allergy to any of the vaccine constituents.

Study Vaccines

GlaxoSmithKline, Quebec, Canada, manufactured all vaccines. The TIV (*FluLaval*™ 2009–2010) was an inactivated split-virion influenza vaccine that contained 45 μg hemagglutinin (HA) antigen/0.5-mL dose (15 μg HA of each of 3 strains [A/Brisbane/59/2007(H1N1) IVR-148, A/Uruguay/716/2007(H3N2) NYMCX-175C, and B/Brisbane/60/2008(B)]. The placebo was phosphate-buffered saline (PBS; 0.5-mL dose).

The A(H1N1)pdm09 vaccine was a monovalent split-virion influenza vaccine that contained HA of A/California/7/2009 (H1N1) NYMC X-179A mixed with either PBS to contain 15 μg HA/0.5-mL dose (groups B and D) or with $AS03_A$ to contain 3.75 μg HA/0.5-mL dose (groups A and C). $AS03_A$ (henceforth referred to as AS03) is an oil-in-water emulsion-based adjuvant system that contains 11.86 mg tocopherol/dose. Vaccines and placebo were administered intramuscularly (deltoid) in the nondominant arm (doses 1 and 2) or dominant arm (dose 3).

Immunogenicity Evaluations

Immunogenicity evaluations were performed at each blood-sampling time point with the exception of visit days 14 and 125 for NAb responses and visit day 125 for HI responses.

Assessment of Humoral Immune Responses

Neutralization and HI assays were performed as described previously [12, 31]. HI responses were assessed using seropositivity rates (percentages of participants with titer ≥1:10), seroconversion rates (SCRs; percentage of participants with prevaccination titer <1:10 and postvaccination titer ≥1:40 or prevaccination

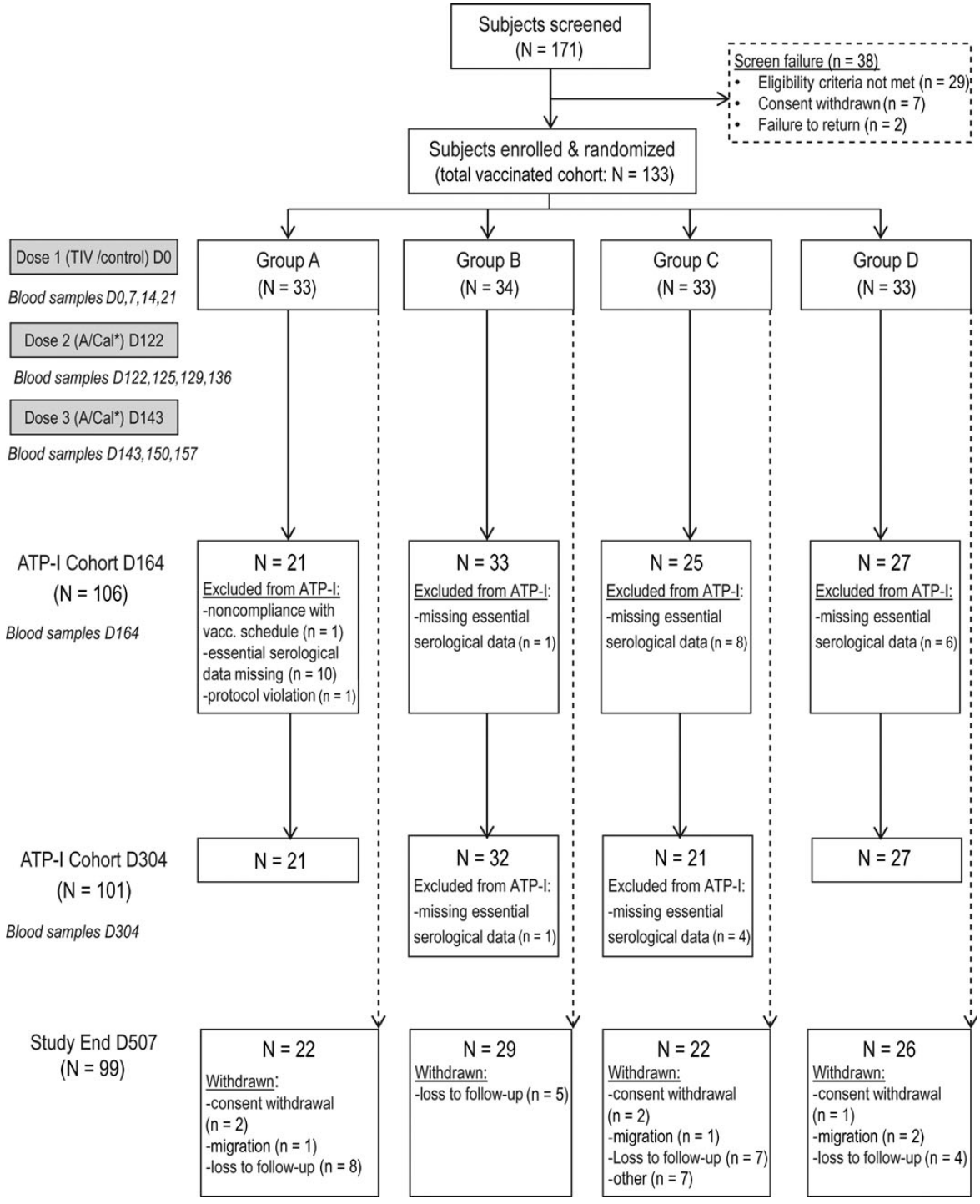

Figure 1. CONSORT diagram of study flow. A/Cal*, A(H1N1)pdm09 vaccine. Group A received trivalent seasonal influenza vaccine (TIV) and 2 doses of A(H1N1)pdm09 (3.75 μg) /AS03. Group B received TIV and 2 doses of A(H1N1)pdm09 (15 μg). Group C received saline and 2 doses of A(H1N1)pdm09 (3.75 μg) /AS03. Group D received saline and 2 doses of A(H1N1)pdm09 (15 μg). Abbreviations: D, day; N, number of participants who received the vaccine and for whom data were available.

titer >1:10 and ≥4-fold increase in postvaccination titer, with day 122 considered as prevaccination for day 304), seroprotection rates (SPRs; percentage of participants with titer ≥1:40), and geometric mean fold rises (GMFRs; geometric mean of the within-participant ratios of postvaccination reciprocal titer to prevaccination reciprocal titer at days 0 or 122). Assessment

of HI responses was based on the European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP) guidance targets for pandemic influenza vaccines in adults [32] (point estimates, SCR >40%; SPR >70%; GMFR >2.5) and the Center for Biologics Evaluation and Research (CBER) licensure criteria [33] [lower limits of 95% confidence interval (CI) ≥40% for SCR and ≥70% for SPR]. Seropositivity rate for NAb responses was the percentage of participants with titer ≥1:28.

Assessment of T-Cell and B-Cell Responses

T-cell frequencies were evaluated using intracellular cytokine staining (ICS) and flow cytometry, as described previously [9]. B-cell frequencies were assessed by memory B-cell enzyme-linked immunosorbent spot (ELISPOT), as described previously [27]. Frequencies of plasmablasts ($CD27^+/CD38^+$ B cells) were evaluated following a previously described method [24] and expressed as percentages of $CD3^-/CD20^{lo}/CD19^+$ B cells.

Safety and Reactogenicity Evaluation

Solicited local and general adverse events (AEs) were recorded during the 7-day period post vaccination (days 0–6, 122–128, 143–149); unsolicited AEs during the 84-day, 42-day, and 21-day periods following doses 1, 2 and 3, respectively; and selected hematological and biochemical clinical laboratory parameter abnormalities on days 0, 21, 122, 164, and 304. The occurrence of/relationship to vaccination of medically attended adverse events (MAEs), potential immune-mediated diseases (pIMDs), serious AEs (SAEs), and withdrawals due to AEs were assessed up to day 507.

Statistical Methods

Descriptive analyses were performed. Immunogenicity analyses were performed on the according-to-protocol cohorts for immunogenicity (ATP-I) at days 164 and 304. Geometric mean titers (GMTs), SCRs, SPRs, and GMFRs for HI antibodies and GMTs and SCRs for NAb responses were tabulated with 95% CIs. Safety analysis was based on the total vaccinated cohort (TVC). Incidences of solicited and unsolicited AEs after each vaccination were tabulated with 95% CIs.

RESULTS

Population Demographics

Overall, 171 participants were screened, of whom 133 (77.8%) were vaccinated and included in the TVC. Ninety-nine patients completed the study at day 507 (Figure 1). Withdrawals from the study (24 participants) were mainly due to loss to follow-up. Demographics were comparable across groups. In the TVC, the median age was 29 years (range, 19–40 as per protocol), the male-to-female ratio was 45.9%:54.1%, and the majority of participants were of white-Caucasian/European (70.7%) or African (27.1%) heritages.

Immunogenicity

A(H1N1)pdm09-Specific HI and NAb Responses

After TIV or placebo administration (dose 1; days 0–122), GMTs of A(H1N1)pdm09-specific HI responses in both groups remained low (ranges, 12.5–19.9 and 13.3–13.9, respectively), and CHMP or CBER criteria were not met (Supplementary Table 1).

At day 122, the day of the first A(H1N1)pdm09 vaccination (dose 2), A(H1N1)pdm09-specific HI antibody responses (GMTs) ranged from 12.5 to 16.1 across groups (Table 1 and Figure 2*A*). Two weeks later (day 136), GMTs had increased substantially (range GMFRs, 24–40 across groups) and, when comparing the adjuvanted groups A and C as well as the non-adjuvanted groups B and D, tended to be lower after previous administration of TIV (GMTs 492, 603, 304, and 386, respectively). There was also a tendency for higher GMTs in the adjuvanted groups relative to nonadjuvanted groups. All participants were A(H1N1)pdm09 seropositive from day 136 onward (14 days following first dose of A(H1N1)pdm09 vaccine [dose 2]).

At day 164, 3 weeks after the second A(H1N1)pdm09 vaccination (dose 3), GMTs had further increased in the adjuvanted groups and remained relatively constant in the nonadjuvanted groups. GMTs in the saline-primed groups still tended to exceed those in the TIV-primed groups for both pandemic vaccine formulations, with this trend being greater for the adjuvanted groups A and C (651 vs 799) than for the nonadjuvanted groups B and D (259 vs 333). Moreover, GMTs were higher following adjuvanted vaccine relative to nonadjuvanted vaccine, with greater differences between the saline-primed groups C and D. Similar trends were observed in SCRs, SPRs, and GMFRs. SCRs were 84.8%–100% across groups, SPRs 100% in all groups, and GMFRs ranged from 20.8 (group B) to 57.4 (group C), thus fulfilling CHMP and CBER criteria. Between day 164 and day 304, GMTs decreased 3- to 4-fold but remained, along with SCRs, SPRs, and seropositivity rates, above baseline (day 122) values in all groups; at day 304 SCRs and SPRs still met CHMP criteria.

NAb responses followed trends that were similar to trends for HI responses, with a tendency for higher responses in saline-primed groups relative to TIV-primed groups ($A < C$ and $B < D$) and in adjuvanted groups relative to nonadjuvanted groups ($A > B$ and $C > D$). Differences between groups were generally smaller than observed for HI responses.

A/Brisbane/59/2007-Specific HI and NAb Responses

At day 21, an A/Brisbane/59/2007-specific HI antibody response was observed only in the TIV group (GMT 200 vs

Table 1. A(H1N1)pdm09-Specific Hemagglutination Inhibition Responses Before and After Vaccination

Group	Day	N	n	Seroconversion Rate, % (95% CI)	n	Seroprotection Rate, % (95% CI)	n	Seropositivity, % (95% CI)	Geometric Mean Fold Rise Value (95% CI)	Geometric Mean Titer Value (95% CI)
A	122	21		NA	7	33.3 (14.6–57.0)	12	57.1 (34.0–78.2)	NA	16.1 (8.9–29.3)
	129	21	9	42.9 (21.8–66)	13	61.9 (38.4–81.9)	20	95.2 (76.2–99.9)	4.1 (2.5–6.7)	66.7 (35.7–124.6)
	136	21	21	100 (83.9–100)	21	100 (83.9–100)	21	100 (83.9–100)	30.5 (17.4–53.3)	491.6 (319.3–756.9)
	143	21	19	90.5 (69.6–98.8)	21	100 (83.9–100)	21	100 (83.9–100)	24.2 (13.6–42.9)	390.1 (266.3–571.5)
	150	19	19	100 (82.4–100)	19	100 (82.4–100)	19	100 (82.4–100)	31.4 (17.4–56.9)	452.8 (305.6–670.9)
	157	20	20	100 (83.2–100)	20	100 (83.2–100)	20	100 (83.2–100)	43.0 (25.3–73.2)	651.3 (487.1–870.8)
	164	21	21	100 (83.9–100)	21	100 (83.9–100)	21	100 (83.9–100)	40.3 (22.9–71.2)	650.7 (485.5–872.1)
	304	21	16	76.2 (52.8–91.8)	19	90.5 (69.6–98.8)	21	100 (83.9–100)	NA	157.4 (98.2–252.2)
B	122	33		NA	7	21.2 (9.0–38.9)	14	42.4 (25.5–60.8)	NA	12.5 (7.7–20.1)
	129	31	9	29.0 (14.2–48.0)	17	54.8 (36.0–72.7)	26	83.9 (66.3–94.5)	4.2 (2.3–7.6)	54.6 (28.7–103.9)
	136	33	26	78.8 (61.1–91.0)	30	90.9 (75.7–98.1)	33	100 (89.4–100)	24.4 (13.3–44.8)	303.7 (191.7–481.0)
	143	33	26	78.8 (61.1–91.0)	31	93.9 (79.8–99.3)	33	100 (89.4–100)	20.6 (11.4–37.2)	256.6 (164.9–399.3)
	150	31	26	83.9 (66.3–94.5)	30	96.8 (83.3–99.9)	31	100 (88.8–100)	22.6 (12.9–39.8)	292.7 (198.0–432.7)
	157	32	27	84.4 (67.2–94.7)	32	100 (89.1–100)	32	100 (89.1–100)	23.6 (14.0–39.8)	303.2 (215.1–427.4)
	164	33	28	84.8 (68.1–94.9)	33	100 (89.4–100)	33	100 (89.4–100)	20.8 (12.5–34.6)	259.4 (186.4–361.0)
	304	32	21	65.6 (46.8–81.4)	28	87.5 (71.0–96.5)	32	100 (89.1–100)	NA	100.3 (68.0–148.2)
C	122	25		NA	6	24.0 (9.4–45.1)	13	52.0 (31.3–72.2)	NA	13.9 (8.1–24.0)
	129	25	14	56.0 (34.9–75.6)	18	72.0 (50.6–87.9)	23	92.0 (74.0–99.0)	6.0 (3.4–10.4)	83.3 (44.2–157.3)
	136	23	22	95.7 (78.1–99.9)	23	100 (85.2–100)	23	100 (85.2–100)	39.6 (21.7–72.1)	602.5 (434.1–836.4)
	143	25	24	96.0 (79.6–99.9)	25	100 (86.3–100)	25	100 (86.3–100)	40.0 (22.1–72.4)	557.3 (426.5–728.3)
	150	25	24	96.0 (79.6–99.9)	25	100 (86.3–100)	25	100 (86.3–100)	40.6 (22.6–72.8)	565.0 (423.3–754.1)
	157	24	23	95.8 (78.9–99.9)	24	100 (85.8–100)	24	100 (85.8–100)	56.3 (30.7–103.3)	818.1 (634.9–1054.0)
	164	25	25	100 (86.3–100)	25	100 (86.3–100)	25	100 (86.3–100)	57.4 (31.6–104.2)	799.0 (630.5–1012.4)
	304	21	16	76.2 (52.8–91.8)	21	100 (83.9–100)	21	100 (83.9–100)	NA	289.9 (188.2–446.6)
D	122	27		NA	4	14.8 (4.2–33.7)	13	48.1 (28.7–68.1)	NA	12.9 (8.1–20.7)
	129	27	17	63.0 (42.4–80.6)	20	74.1 (53.7–88.9)	23	85.2 (66.3–95.8)	5.5 (3.4–8.9)	70.3 (38.8–127.6)
	136	26	25	96.2 (80.4–99.9)	26	100 (86.8–100)	26	100 (86.8–100)	28.8 (16.7–49.9)	385.6 (242.9–612.1)
	143	26	24	92.3 (74.9–99.1)	26	100 (86.8–100)	26	100 (86.8–100)	27.3 (15.8–47.3)	365.6 (241.3–553.9)
	150	26	24	92.3 (74.9–99.1)	26	100 (86.8–100)	26	100 (86.8–100)	24.9 (14.9–41.6)	333.3 (220.8–503)
	157	25	23	92.0 (74.0–99.0)	25	100 (86.3–100)	25	100 (86.3–100)	24.3 (14.8–40.0)	338.2 (230.7–495.7)
	164	27	26	96.3 (81.0–99.9)	27	100 (87.2–100)	27	100 (87.2–100)	25.8 (15.9–41.8)	332.7 (222.0–498.6)
	304	27	17	63.0 (42.4–80.6)	23	85.2 (66.3–95.8)	27	100 (87.2–100)	NA	116.0 (72.7–185.2)

Data shown are of the according-to-protocol cohort for immunogenicity (ATP-I cohort) at day 164, except for day 304, which are for the ATP-I cohort at day 304. Groups A and B received trivalent seasonal influenza vaccine at day 0 and either adjuvanted (group A) or nonadjuvanted (group B) pandemic vaccine at day 122 and day 143. Groups C and D received saline at day 0 and adjuvanted (group C) or nonadjuvanted (group D) pandemic vaccine at day 122 and day 143. Seroconversion rate: n/%, = number/percentage of seroconverted participants. Seroconversion: titer ≥40 1/DIL after vaccination (for initially seronegative participants at day 0) or a titer after vaccination ≥4-fold the prevaccination titer (for initially seropositive participants at day 0). Seropositivity: n/%, number/percentage of participants with titer within the specified range. Seroprotection rate: n/%, number/percentage of participants with titer within the specified range. Geometric mean fold rise: geometric mean of the within-participant ratios of the post-vaccination reciprocal HI titer to the day 122 reciprocal HI titer before pandemic vaccination. Abbreviations: CI, confidence interval; NA, not available.

18.8 for the saline group), with SPRs meeting CHMP and CBER criteria (Supplementary Table 1).

After A(H1N1)pdm09 vaccine administration (doses 2 and 3; days 129–304), GMTs in the TIV-primed groups remained at levels comparable to prevaccination levels (day 122; Supplementary Table 2 and Figure 2*B*). In the saline-primed groups, GMTs increased slightly but remained lower than in the TIV-primed groups. Irrespective of the priming, GMTs in the adjuvanted groups were comparable to those in the nonadjuvanted groups. At day 164, SCRs met the CHMP criterion in both TIV-primed groups A and B but not in the saline-primed groups C and D (76.2%, 54.5%, 24%, and 7.4%, respectively), while they met the CBER criterion only in group A. SPRs met the CHMP criterion in groups A–C at day 164 and day 304 (range, 72%–95.2%) but not in group D (51.9% on both days), while the CBER criterion was only met in both TIV-primed groups. The CHMP criterion for GMFR was met at day 164 in all groups except group D.

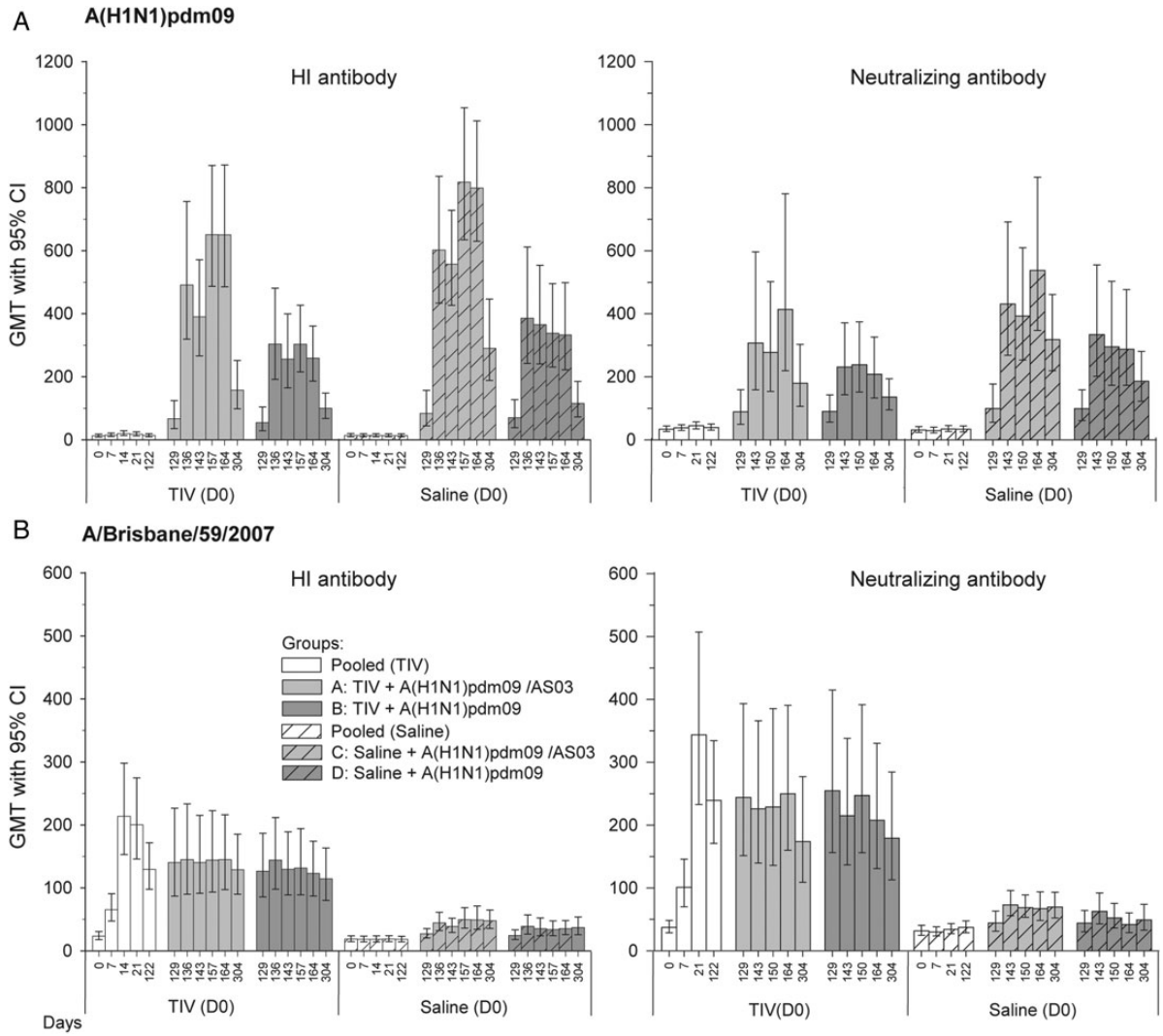

Figure 2. A(H1N1)pdm09-specific and A/Brisbane/59/2007-specific hemagglutination inhibition (HI) and neutralizing antibody (NAb) responses. A(H1N1)pdm09-specific (panel *A*) and A/Brisbane/59/2007-specific (panel *B*) HI and NAb responses shown are of the according-to-protocol cohort for immunogenicity (ATP-I cohort) at D164, except those for day 304 (from ATP-I cohort at day 304). Group A received trivalent seasonal influenza vaccine (TIV) at day 0 and A(H1N1)pdm09 (3.75 μg)/AS03 at day 122 and day 143. Group B received TIV at day 0 and A(H1N1)pdm09 (15 μg) at day 122 and day 143. Group C received saline at day 0 and A(H1N1)pdm09 (3.75 μg)/AS03 at day 122 and day 143. Group D received saline at day 0 and A(H1N1)pdm09 (15 μg) at day 122 and day 143. Abbreviations: CI, confidence interval; GMT, geometric mean titer.

As compared with HI responses, NAb responses followed parallel trends (Figure 2*B*).

B-Cell Responses

We evaluated the roles of adjuvant and TIV priming in inducing B-cell responses. First, frequencies of A(H1N1)pdm09-specific memory B cells were measured by ELISPOT (Figure 3*A*). Low frequencies were observed prior to vaccination (day 0) in all groups. Some A(H1N1)pdm09-specific memory B-cell responses were induced by the TIV. Following vaccination with A(H1N1)pdm09 vaccines, stronger memory B-cell responses were observed with adjuvanted vaccine relative to non-adjuvanted vaccine (groups: A > B and C > D). Moreover, TIV vaccination prior to adjuvanted A(H1N1)pdm09 vaccination had a negative effect on the memory B-cell frequencies (group: C > A).

We also evaluated the frequencies of plasmablasts (defined as $CD27^+/CD38^+$ B cells) as a percentage of B cells ($CD20^{lo}/CD19^+/CD3^-$; days 0–129; Figure 3*B*). It has previously been shown that plasmablasts can be detected 7 days after TIV vaccination and that a large proportion of $CD27^+/CD38^+$ plasmablasts are specific for influenza vaccine antigens [24]. Evaluation of $CD27^+/CD38^+$ plasmablast frequencies 1 week after dose 1 revealed increased frequencies in both TIV groups but not in the saline groups. One week post A(H1N1)pdm09 vaccination (day 129), plasmablast responses, defined

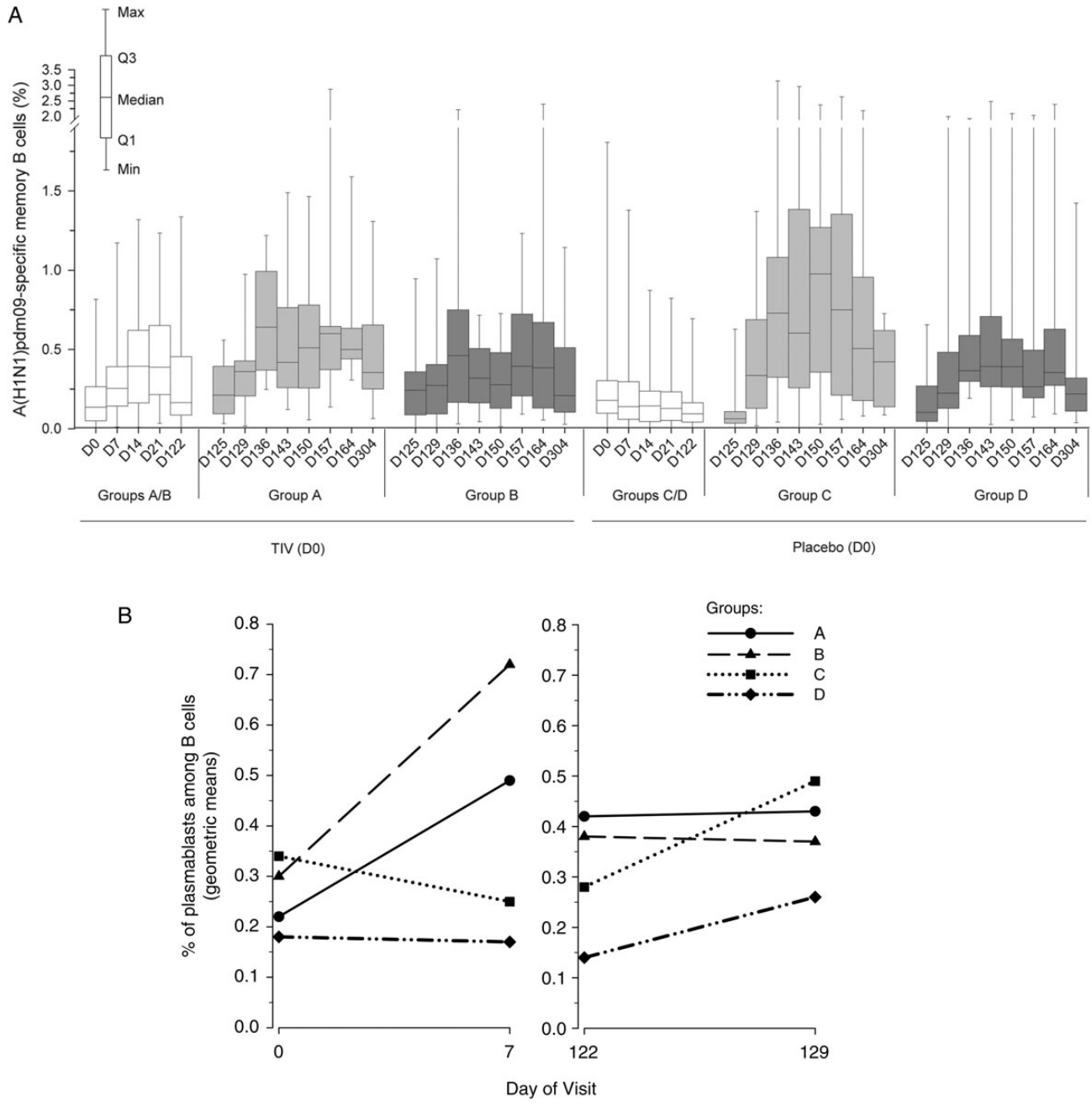

Figure 3. A(H1N1)pdm09-specific memory B-cell responses to A(H1N1)pdm vaccine and proportion of plasmablasts ($CD27^+/CD38^+$) among B cells ($CD19^+/CD3^-$) after trivalent seasonal influenza vaccine (TIV) vaccination. *A*, Data shown are of the according-to-protocol cohort for immunogenicity at day 304. Group A received TIV at day 0 and A(H1N1)pdm09 (3.75 μg)/AS03 at day 122 and day 143. Group B received TIV at day 0 and A(H1N1)pdm09 (15 μg) at day 122 and day 143. Group C received saline at day 0 and A(H1N1)pdm09 (3.75 μg)/AS03 at day 122 and day 143. Group D received saline at day 0 and A(H1N1)pdm09 (15 μg) at day 122 and day 143. Data are reported as the percentages of A/California/7/2009-specific memory B cells of all B cells, with first and third quartiles, and the minimum/maximum values measured. *B*, Geometric means of plasmablast ($CD27^+/CD38^+$) frequencies expressed as a percentage of B cells ($CD20^{lo}/CD19^+/CD3^-$) after administration of TIV (days 0–129). Abbreviation: D, day.

by increased frequencies of $CD27^+/CD38^+$ B cells, were observed in both saline-primed groups but not in the TIV-primed groups, with the strongest increase in the (adjuvanted) group C.

T-Cell Responses

In order to evaluate the effects of the adjuvant on the frequency and quality (polyfunctionality) of A(H1N1)pdm09-specific and TIV strain–specific T-cell responses, we assessed the vaccine-

induced responses of antigen-specific $CD4^+$ T cells expressing at least 2 immune markers (among CD40L, interleukin [IL]-2, tumor necrosis factor-alpha [TNF-α], and interferon-gamma [IFN-γ]) by ICS after in vitro stimulation of peripheral blood mononuclear cells.

Low responses of A(H1N1)pdm09-specific immune marker–expressing cells were seen after TIV administration but not after administration of saline (Figure 4*A*). After pandemic vaccination, A(H1N1)pdm09-specific $CD4^+$ T-cell responses tended to be lower in the TIV-primed group A than in the saline-primed group C among the adjuvanted groups and were of comparable magnitudes for saline and TIV-primed groups among nonadjuvanted groups. Responses to the adjuvanted vaccine tended to be higher and more persistent, regardless of previous administrations of TIV or saline (groups: A > B and C > D). $CD4^+$ T-cell responses specific for the TIV strains after pandemic vaccination followed similar patterns, although with smaller differences between groups (shown for A/Brisbane/59/2007; Figure 4*B*).

Cytokine expression profiles of responding $CD4^+$ T cells were assessed by evaluating the frequencies of antigen-specific cells producing at least 1 marker (Supplementary Figure 1; shown for A(H1N1)pdm09). Different cytokines were produced, with more cells expressing IL-2 and/or CD40L than TNF-α and/or IFN-γ. The strongest responses were measured in the adjuvanted groups A and C.

There were no detectable $CD8^+$ T-cell responses to the A(H1N1)pdm09 strain or any of the TIV strains (data not shown).

Safety and Reactogenicity

Solicited AEs

Following dose 1, 65.7% and 28.8% of the recipients of TIV and saline, respectively, reported any symptom. Among local symptoms, injection site pain was most frequently reported and was more common in the TIV group than in the saline group (53% vs 7.8%; Figure 5). Among general symptoms, headache, joint pain, and muscle aches were also more common among TIV recipients, and grade 3 AEs were only reported in the TIV group (1 each of fatigue, headache, muscle aches, and joint pain).

After A(H1N1)pdm09 vaccination (doses 2/3), injection site pain was also the most frequently reported local AE and occurred at a higher rate in the adjuvanted groups A and C than in the nonadjuvanted groups B and D (76.0%, 82.8%, 43.8%, and 37.0%, respectively), while no clear trend was observed for grade 3 pain. Fatigue, muscle aches, and headache were the most frequently reported general AEs. Grade 3 general AEs occurred at a frequency of ≤10.3% of participants' AEs.

Unsolicited AEs

After dose 1, unsolicited AEs occurred at similar frequencies in TIV and placebo recipients; 22.4% and 21.2% of participants, respectively, reported at least 1 unsolicited AE. Grade 3 back pain was reported once by a placebo recipient. Nine percent of all participants (10.4% and 7.6% in TIV and saline groups, respectively) reported at least 1 MAE.

After A(H1N1)pdm09 vaccination (doses 2 and 3), frequencies of unsolicited AEs were comparable among groups (Table 2). Nausea and upper respiratory tract infections were the most common events (≤2 participants/group). Two grade 3 AEs (diabetes mellitus and uterine leiomyoma) were reported in group A. From the participants of groups A–D, 27.3%, 32.4%, 15.2%, and 12.1%, respectively, experienced at least 1 MAE up to day 507, none of which were considered related to vaccination, and 9.1%, 2.9%, 3.0%, and 3.0%, respectively, experienced at least 1 SAE, none of which were fatal or considered related to vaccination. SAEs (1 report each) included hepatitis C 7 days after dose 1, which was unresolved at day 507; uterine fibroid that developed within 1 month after dose 3; worsening of aortic aneurysm 37 days after dose 3 (all in group A); elevated liver-function test 6 months after dose 3 (group B); moderate *Clostridium difficile* infection 63 days after dose 3 (group C); and cholelithiasis 5 months after dose 3 (group D).

There were no reports of pIMDs or major clinical laboratory abnormalities, and there were no withdrawals due to SAEs or AEs.

DISCUSSION

This study assessed the effect of prior vaccination with a TIV on the immune response to the A(H1N1)pdm09 vaccine in young, healthy adult volunteers lacking a history of previous influenza vaccination. Secondary objectives were to assess the role of the adjuvant on CMI and humoral responses and to evaluate vaccine safety.

Although no formal statistical comparison between groups was made, our results indicate that there was a trend for diminished A(H1N1)pdm09-specific humoral and $CD4^+$ T-cell responses following vaccination with the pandemic vaccine in participants who had previously received TIV. Also, results showed that adjuvantation of the A(H1N1)pdm09 vaccine led to increased responses of vaccine-homologous and -heterologous HI antibodies, NAb and $CD4^+$ T cells, and homologous memory B cells and plasmablasts.

Receipt of TIV a few weeks to many months prior to A(H1N1)pdm09 or H5N1 vaccines has previously been shown to affect HI responses [5, 8–20], irrespective of whether these vaccines were adjuvanted or not. One explanation could be the behavior of the memory B-cell pool after vaccination. Seasonal vaccination has been shown to lead to rapid expansion of plasmablasts that produce vaccine antigen-specific antibodies [24]. The B-cell response to the TIV could be shaped by the epitopes present on the TIV strains. The resulting B-cell memory pool could limit the capacity of the B-cell compartment to adapt to antigenically more distant vaccines, such as A(H1N1)pdm09

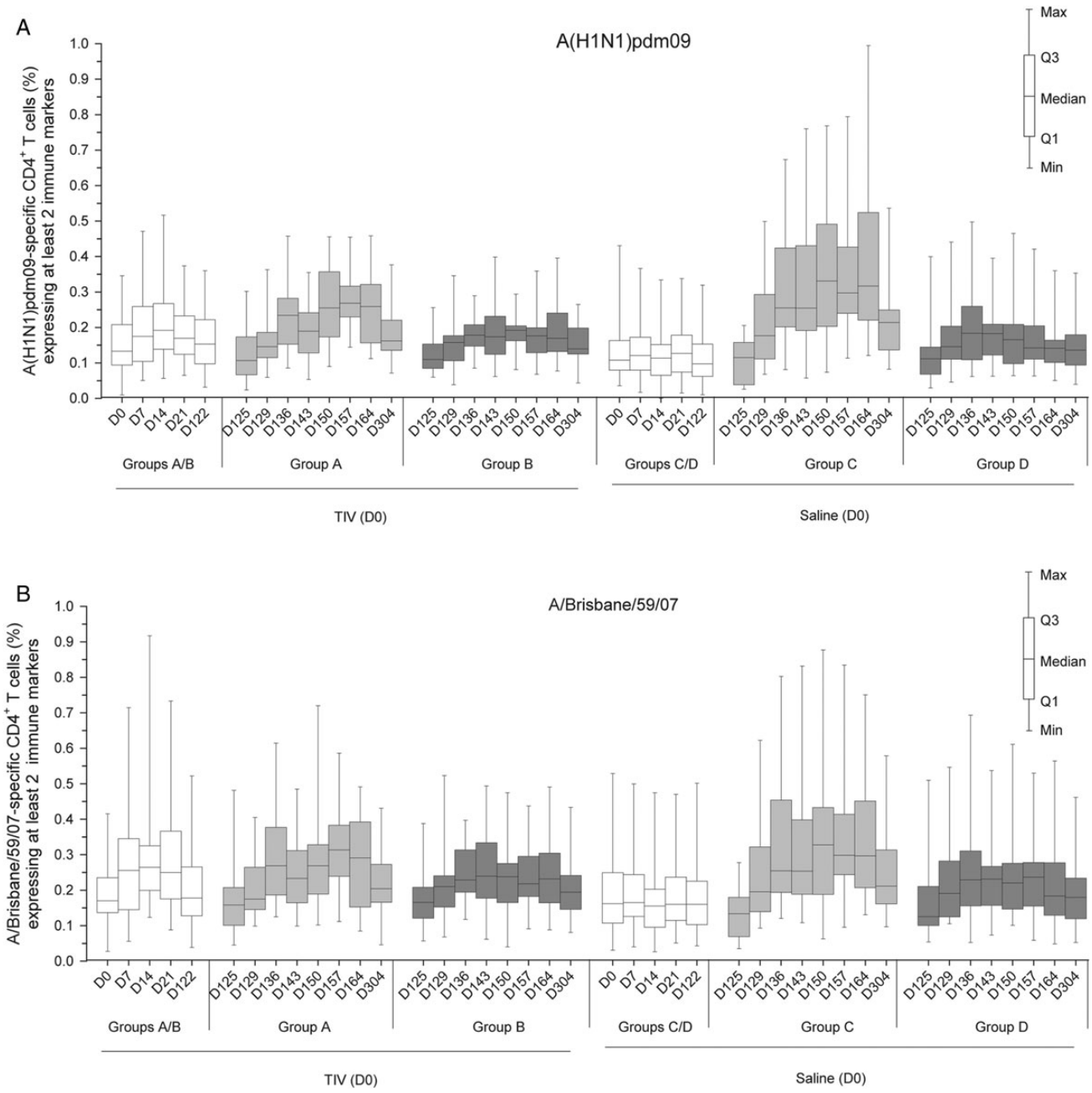

Figure 4. Frequency of A(H1N1)pdm09-specific and A/Brisbane/59/2007-specific $CD4^+$ T cells expressing at least 2 immune markers. Data shown are of the according-to-protocol cohort for immunogenicity at day 304. Group A received trivalent seasonal influenza vaccine (TIV) at day 0 and A(H1N1)pdm09 (3.75 µg)/AS03 at day 122 and day 143. Group B received TIV at day 0 and A(H1N1)pdm09 (15 µg) at day 122 and day 143. Group C received saline at day 0 and A(H1N1)pdm09 (3.75 µg)/AS03 at day 122 and day 143. Group D received saline at day 0 and A(H1N1)pdm09 (15 µg) at day 122 and day 143. Data are reported as the percentages of A(H1N1)pdm09-specific (*A*) or A/Brisbane/59/2007-specific (*B*) $CD4^+$ T cells expressing (after in vitro stimulation) at least 2 immune markers among interferon-gamma, interleukin-2, tumor necrosis factor-alpha, and CD40L of all $CD4^+$ T cells, with first and third quartiles, and the minimum/maximum values measured. Abbreviation: D, day.

vaccine antigens administered subsequently. Thus, a B-cell repertoire that is "fixed" by TIV could limit adaptability of this response. $CD4^+$ T cells can have a role in helping memory B cells by stimulating somatic hypermutation, thereby facilitating adaptability of the B-cell response [34]. Indeed, not only frequencies of specific $CD4^+$ T cells but also HI responses and memory B-cell frequencies were enhanced after the first and second doses of adjuvanted vaccine; this is in line with observations in previous H5N1 and/or A(H1N1)pdm09 vaccine studies [9, 27]. Moreover, the epitope breadth and binding

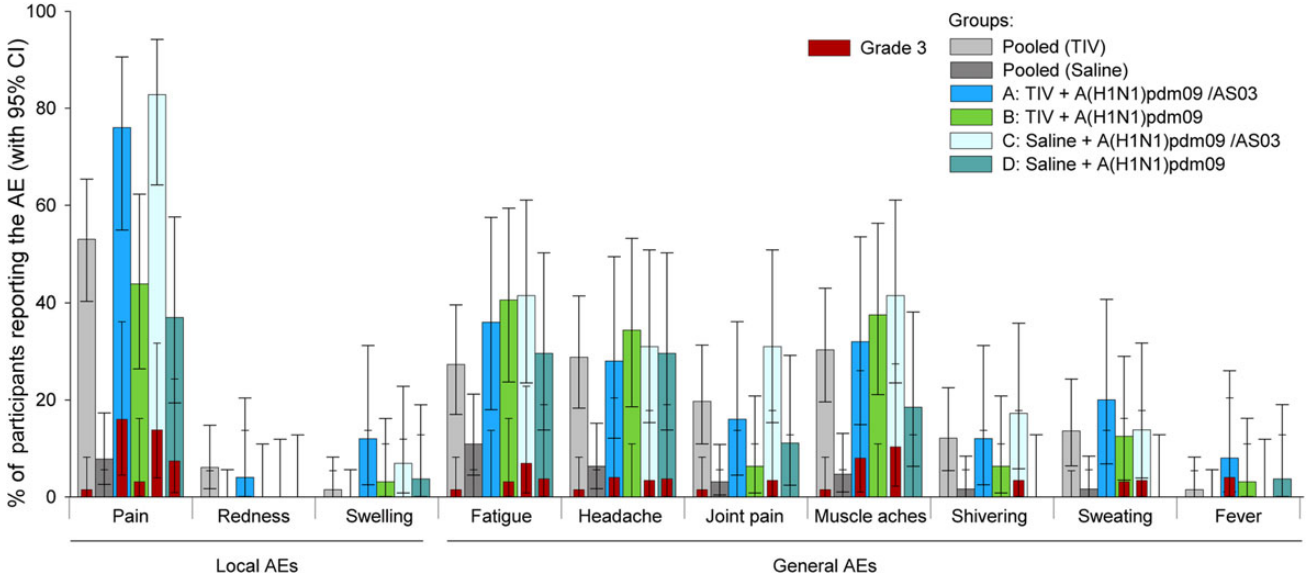

Figure 5. Incidence of solicited symptoms after the administration of trivalent seasonal influenza vaccine (TIV) or placebo and after each of the 2 doses of A(H1N1)pdm09 vaccine. Mean percentage of participants with 95% confidence interval (CI) experiencing solicited local (*A*) and general (*B*) adverse events (AEs) reported within 7 days postvaccination are shown for all participants for whom at least 1 administration of vaccine or placebo was documented (the total vaccinated cohort). Group A received TIV at day 0 and A(H1N1)pdm09 (3.75 μg)/AS03 at day 122 and day 143. Group B received TIV at day 0 and A (H1N1)pdm09 (15 μg) at day 122 and day 143. Group C received saline at day 0 and A(H1N1)pdm09 (3.75 μg)/AS03 at day 122 and day 143. Group D received saline at day 0 and A(H1N1)pdm09 (15 μg) at day 122 and day 143.

affinity of the antibodies to pandemic influenza vaccines were previously shown to be improved by MF59, another oil-in-water–based adjuvant [28, 29].

In a previous A(H1N1)pdm09 vaccine study, $CD4^+$ T-cell frequencies after the first dose of pandemic vaccine correlated with HI titers measured 3 weeks later [9]. Although the presence of such correlation was not assessed in our study, we speculate that after the first dose of adjuvanted vaccine, stimulation of $CD4^+$ T-cell responses may have resulted in increased "help" for B cells, resulting in better adaptation of B cells and, subsequently, in increased HI titers to the variant HA in the pandemic vaccine. In short, after the first dose of pandemic vaccine, the adjuvant may have promoted B-cell adaptation to the more distant A(H1N1)pdm09 antigen and helped to shape T- and B-cell pools to better respond to the subsequent vaccination.

Overall, the reactogenicity and safety data for the TIV recipients were consistent with data for those who received a comparable TIV [35]. Injection site pain was more common in the TIV

Table 2. Unsolicited Adverse Events, >1% Incidence Overall, Following the 21-Day Period After Each Dose of A(H1N1)pdm09 Vaccine

	Group A (N = 28)		Group B (N = 33)		Group C (N = 29)		Group D (N = 28)	
Adverse Event	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
≥1 adverse event	8	28.6 (13.2–48.7)	6	18.2 (7.0–35.5)	7	24.1 (10.3–43.5)	5	17.9 (6.1–36.9)
Nausea	1	3.6 (0.1–18.3)	0	0.0 (0.0–10.6)	2	6.9 (0.8–22.8)	1	3.6 (0.1–18.3)
Vomiting	0	0.0 (0.0–12.3)	0	0.0 (0.0–10.6)	1	3.4 (0.1–17.8)	1	3.6 (0.1–18.3)
Nasopharyngitis	1	3.6 (0.1–18.3)	1	3.0 (0.1–15.8)	0	0.0 (0.0–11.9)	0	0.0 (0.0–12.3)
Sinusitis	1	3.6 (0.1–18.3)	1	3.0 (0.1–15.8)	0	0.0 (0.0–11.9)	0	0.0 (0.0–12.3)
Upper respiratory tract infection	0	0.0 (0.0–12.3)	1	3.0 (0.1–15.8)	1	3.4 (0.1–17.8)	1	3.6 (0.1–18.3)
Oropharyngeal pain	1	3.6 (0.1–18.3)	0	0.0 (0.0–10.6)	1	3.4 (0.1–17.8)	0	0.0 (0.0–12.3)
Rhinorrhea	1	3.6 (0.1–18.3)	0	0.0 (0.0–10.6)	1	3.4 (0.1–17.8)	0	0.0 (0.0–12.3)

Data shown are of the total vaccinated cohort. Groups A and B received trivalent seasonal influenza vaccine at day 0 and either adjuvanted (group A) or nonadjuvanted (group B) pandemic vaccine at day 122 and day 143. Groups C and D received saline at day 0 and adjuvanted (group C) or nonadjuvanted (group D) pandemic vaccine at day 122 and day 143. Abbreviations: CI, confidence interval; N, number of participants with the administered dose; n/%, number/percentage of participants reporting the symptom at least once.

group than in the placebo group after first vaccination and in recipients of adjuvanted vaccine relative to recipients of nonadjuvanted vaccine after A(H1N1)pdm09 vaccination. Consistent with earlier studies with adjuvanted and nonadjuvanted A(H1N1)pdm09 vaccines [9, 11], the current data do not suggest relevant safety concerns for any of the studied vaccines in the given study population. Several retrospective epidemiological studies have described an association between vaccination with a different A(H1N1)pdm09 vaccine (*Pandemrix*™) and the later onset of narcolepsy in persons aged <21 years as well as in adults [36]. Recent experiments revealed a potential molecular basis for the link, which lies in the HA amino acid sequence of H1N1 [37]. No narcolepsy cases were reported in the current study, though the current trial was not designed to detect rare events.

Trial limitations were the small sample size and relatively large number of withdrawals from the study. The study size selection was a consequence of the complexity of the laboratory assays for this descriptive study, while the number of withdrawals may have been related to the trial duration, weekly blood-sampling frequency over the course of 1 year, and/or the relatively large blood-sample volumes required to perform the detailed immunogenicity assessments.

CONCLUSION

In healthy participants aged 19–40 years, prior vaccination with TIV decreased the humoral and CMI responses to the A(H1N1)pdm09 vaccine. Adjuvantation of the pandemic vaccine helped to overcome the immune interference between influenza vaccines. No clinically relevant safety concerns were observed with either of the study vaccines.

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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Potential conflicts of interest. All authors are employees of the GlaxoSmithKline group of companies. S. R.-G. holds GSK stock options and R. v.d M. and D. V. report receiving restricted shares of the company. R. v.d M. declares that US provisional patent applications have been filed in relation to some of the information discussed in this manuscript. All other authors report no potential conflicts.

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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