A clinical trial of intradermal and intramuscular seasonal influenza vaccination in patients with atopic dermatitis

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Background: Antibody responses to the inactivated seasonal influenza vaccine in patients with atopic dermatitis (AD) have not been carefully characterized.
Objective: The primary objective of this study was to compare antibody responses to intradermal vaccination in participants with moderate/severe AD with those in nonatopic participants. Secondary objectives were to evaluate the effect of route of administration, *Staphylococcus aureus* skin colonization, and disease severity on vaccine response.
Methods: This was an open-label study conducted in the 2012-2013 influenza season at 5 US clinical sites. A total of 360 participants with moderate/severe AD or nonatopic subjects were assessed for eligibility, 347 of whom received intradermal or intramuscular vaccination per label and were followed for 28 days after vaccination. The primary outcome was the difference in the proportion of participants achieving seroprotection (hemagglutination-inhibition antibody titer ≥1:40 on day 28 after vaccination).
Results: Seroprotection rates for influenza B, H1N1, and H3N2 were not different (1) between participants with AD and nonatopic participants receiving intradermal vaccination and (2) between AD participants receiving intradermal and intramuscular vaccination. After intradermal, but not intramuscular, vaccination, participants with AD with *S aureus* colonization experienced (1) lower seroprotection and seroconversion rates and lower hemagglutination-inhibition antibody titer geometric mean fold increase against influenza B and (2) lower seroconversion rates against influenza H1N1 than noncolonized participants with AD.
Conclusion: Participants with AD colonized with *S aureus* exhibited a reduced immune response to influenza vaccination compared with noncolonized participants after intradermal but not intramuscular vaccination. Because most patients with AD are colonized with *S aureus*, intramuscular influenza vaccination should be given preference in these patients. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: *Atopic dermatitis,* Staphylococcus aureus*, eczema, influenza, vaccination, skin, antibody*

Atopic dermatitis (AD) is the most common chronic skin disease, affecting more than 15% of children and persisting into adulthood in half of these patients.[1,2] Patients with AD have a unique predisposition to infection by *Staphylococcus aureus* and herpes simplex virus.[3-6] The National Institutes of Health/National Institute of Allergy and Infectious Diseases–funded Atopic Dermatitis Research Network (ADRN) aims to elucidate mechanisms underlying cutaneous and systemic immunity in patients with AD and to identify biomarkers that characterize groups of patients with AD with and without a history of staphylococcal colonization, history of eczema herpeticum, or both.

Intradermal vaccination in normal skin is more immunogenic than intramuscular vaccination.[7-9] The current knowledge of

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Abbreviations used
AD: Atopic dermatitis
ADRN: Atopic Dermatitis Research Network
GMFI: Geometric mean fold increase
GMR: Geometric mean ratio
HAI: Hemagglutination-inhibition
NJH: National Jewish Health
OR: Odds ratio
SASC: *Staphylococcus aureus* skin colonization
SEB: Staphylococcal enterotoxin B
TSST-1: Toxic shock staph glococol 1

antibody responses to intradermal administration of antigens in patients with AD is unknown, but more than 6 million doses of intradermal seasonal influenza vaccine (personal communication, Dr M. Decker, Sanofi Pasteur) have been administered since it was licensed in the United States in 2011.[10]

In the current study the primary analysis compared the antibody responses to intradermal vaccination against influenza strains B, H1N1, and H3N2 in patients with AD compared with those in nonatopic participants. As secondary analyses, we also compared the antibody responses of participants with moderate/severe AD receiving intradermal versus intramuscular vaccination, antibody responses in participants with AD with and without *Staphylococcus aureus* skin colonization (SASC), sex, and race.

METHODS

Participants aged 18 to 64 years received open-label vaccination at 5 centers (National Jewish Health [NJH], University of Rochester, Oregon Health & Science University, Boston Children's Hospital, and Northwestern University) on approval from their institutional review boards. Participants with AD had active moderate/severe skin lesions per the Rajka-Langeland Severity Score.[11] Nonatopic participants had no personal or first-degree family history of AD, asthma, allergic rhinitis, or food allergy. See the Methods section and Table E1 in this article's Online Repository at www.jacionline.org for inclusion/exclusion criteria and classification method of race and ethnicity.

Participants with moderate/severe AD (hereafter referred to as AD) were randomized 1:1 to receive intradermal or intramuscular administration of the 2012-2013 seasonal influenza vaccine.[12] At NJH, nonatopic participants were randomized 3:2 to intradermal or intramuscular vaccination until 23 participants received intramuscular vaccination. Thereafter, the remaining nonatopic participants at NJH received intradermal vaccination. All nonatopic participants at the remaining centers received intradermal vaccination. The 23 nonatopic participants receiving intramuscular vaccination served as a reference group for exploratory analyses (Fig 1). Stratified block randomization was used to balance sex and AD severity between vaccination routes by clinical site.

Hemagglutination-inhibition (HAI) antibody titers and influenza B–specific IgG_1, IgG_2, IgG_3, and IgA by means of ELISA were measured before vaccination and 28 ± 7 days after vaccination. IgE and IgG antibodies specific for toxic shock staph glococol 1 (TSST-1) and staphylococcal enterotoxin B (SEB), total IgE levels, and complete blood counts were measured before vaccination. Prior measurements of total IgE levels and complete blood counts obtained within 30 days of vaccination were used, if available.

S aureus cultures of skin swabs had been obtained previously in nonatopic participants and participants with AD as part of the ADRN Registry. In participants with AD, skin swabs were collected from the participant's most severe AD lesion and also from adjacent nonlesional skin. Methodologies of *S aureus* culture and laboratory assays are presented in the Methods section in this article's Online Repository. Sensitivity analyses involving SASC were also performed for 2 subgroups: (1) including only participants who had an *S aureus* culture within 30 days of the vaccination date or (2) including only participants with moderate disease.

For each of the 3 influenza strains, the primary outcome was the proportion of participants achieving seroprotection (HAI antibody titer ≥ 1:40 on day 28 after vaccination). Secondary outcomes included the geometric mean fold increase (GMFI) in HAI antibody titers from baseline to day 28 after vaccination and the proportion of participants experiencing seroconversion (≥4-fold increase in baseline HAI antibody titers on day 28 after vaccination). Participants with baseline HAI titers of 1:40 or greater for a particular strain were excluded from the analyses for that particular strain, and counts of those not seroprotected at baseline per strain are included in Fig 1.

Demographics and baseline characteristics were compared by using the Fisher exact test for categorical measures and the Wilcoxon 2-sample test for continuous measures. Binary rates are presented as proportions and exact 95% CIs, and comparisons are summarized by using odds ratios (ORs) and the Fisher exact test. Continuous variables were summarized with unadjusted geometric means and 95% CIs. Robust regression models using M-estimation were used to analyze continuous outcomes of $\log_2$ HAI titer fold increase and $\log_{10}$ influenza B-specific IgG_1, IgG_2, IgG_3, and IgA levels. Geometric mean ratios (GMRs) were defined as the ratio of geometric means of one group to the other. Multiple imputation methodology was used for influenza B–specific IgG_1, IgG_2, IgG_3, and IgA levels outside the limits of quantification. Baseline $\log_{10}$ IgE and IgG antibodies specific for TSST-1 and SEB were analyzed by using left-censored Tobit regression models. All continuous models adjust for age and sex. The individual effects of SASC and disease severity were analyzed by using an R_n^2 test[13] from a similar robust regression model, as described above, that included both SASC and disease severity as covariates.

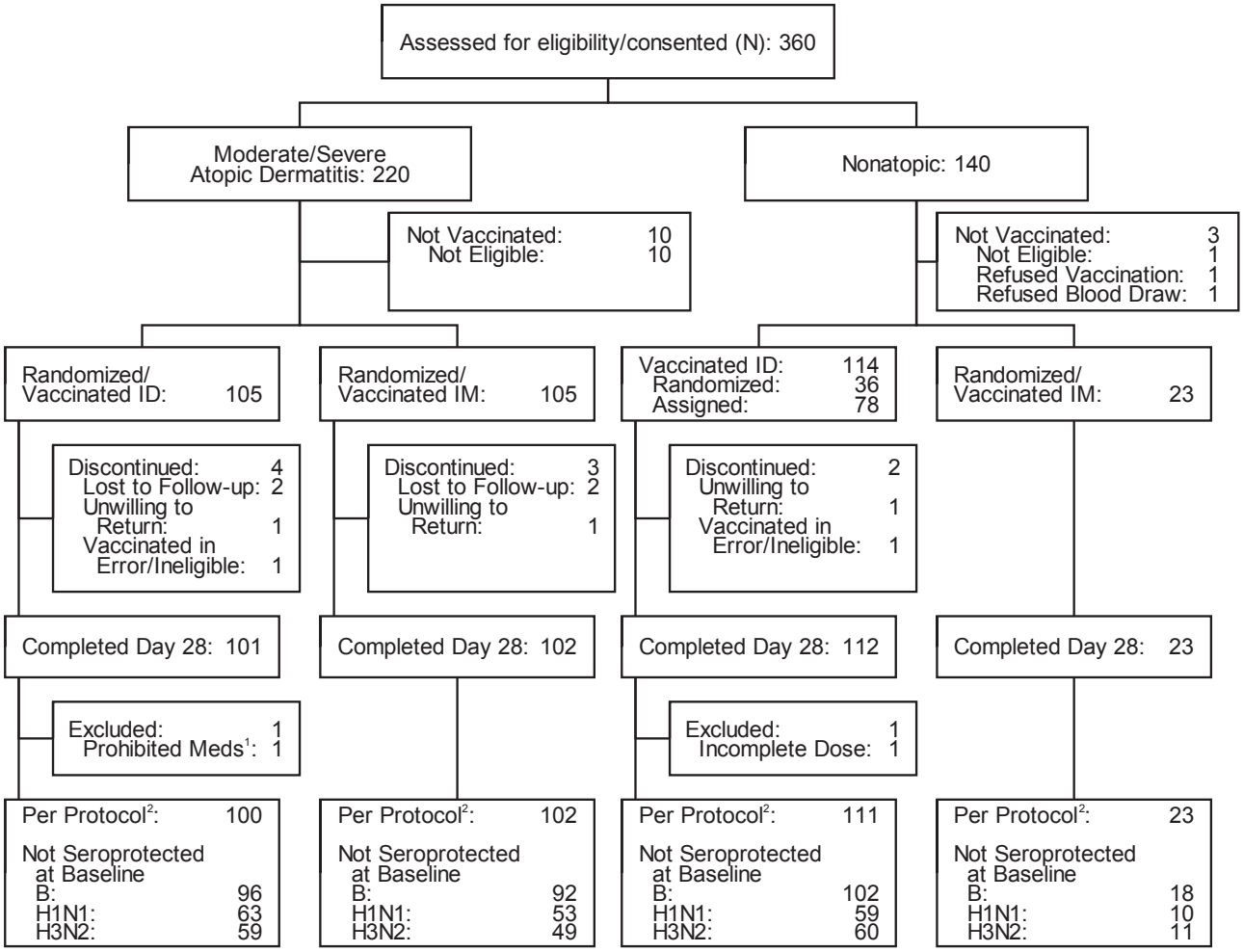
Sample size calculations were based on H3N2 data from our previous ADRN Influenza Vaccine Pilot Study (NCT01518478)[14] with the intradermal 2011-2012 seasonal influenza vaccine,[15] in which 57% and 85% of participants with AD and nonatopic participants, respectively, achieved seroprotection after vaccination. Because no adjustments were made for multiple comparisons among groups or endpoints, all *P* values reported are descriptive/hypothesis generating except for the (inferential) *P* value testing H3N2 seroprotection of participants with AD vs nonatopic participants among those given intradermal vaccination.

By using the Fisher exact test and assuming a 2-sided significance level of .05, a sample size of at least 62 nonatopic participants and 62 participants with AD who were not seroprotected at baseline was necessary to detect a 28% difference in seroprotection rates between participants with AD and nonatopic participants receiving intradermal vaccination with at least 90% power. For secondary objective analyses, we similarly chose a sample size of at least 62 participants with AD without seroprotection at baseline to receive intramuscular vaccination.

RESULTS

Demographics and baseline characteristics

Of 360 candidates screened, 347 were enrolled and vaccinated, and 336 were evaluable in the per-protocol analysis (participants with AD receiving intradermal vaccine, 100; participants with AD receiving intramuscular vaccine, 102; nonatopic participants receiving intradermal vaccine, 111; and nonatopic participants receiving intramuscular vaccine, 23; Fig 1). A total of 136 (43%) of the 313 participants in the 3 main study groups (participants with AD receiving intradermal vaccine, participants with AD receiving intramuscular vaccine, and nonatopic participants receiving intradermal vaccine) were enrolled and vaccinated at NJH. The proportions of the 3 main study groups enrolled at each site were similar across all sites, except Boston Children's Hospital, where nonatopic participants given intradermal vaccination comprised 65% of its enrollment. Among participants receiving intradermal vaccination, the age of the nonatopic group was higher than that of the AD group (Table I). The AD group



[1] Methotrexate (not allowed during the study)

[2] The per protocol population includes participants who 1) received a full dose of vaccine, 2) provided serum samples at baseline and day 28, 3) met eligibility criteria, 4) received no prohibited medications, and 5) had no major protocol deviations.

FIG 1. CONSORT diagram of study participants. *ID,* Intradermal; *IM,* intramuscular.

given intradermal vaccination was not different in sex, race, and ethnicity to either the AD group given intramuscular or the nonatopic group given intradermal vaccination. Among recipients of intradermal vaccination, the nonatopic participant group had lower total IgE levels, eosinophil counts, and proportions of SASC than the AD group. These 3 characteristics were similar between both participants with AD receiving intradermal and those receiving intramuscular vaccination.

Baseline severity measures of AD, such as the Eczema Area and Severity Index and the Rajka-Langeland Severity Score, were similar in participants given intradermal and intramuscular vaccination.

Baseline seroprotection rates were similar between the AD and nonatopic groups for each influenza strain; however, baseline seroprotection rates were low for influenza B compared with H1N1 or H3N2 (see Fig E1 in this article's Online Repository at www.jacionline.org).

Comparative antibody responses of nonatopic participants and participants with AD after intradermal vaccination

There were no differences in seroprotection, seroconversion, or HAI titer GMFI after intradermal vaccination for either influenza B, H1N1, or H3N2 between the nonatopic participants and participants with AD overall (Table II).

Comparative antibody responses of participants with AD after intradermal or intramuscular vaccination

Seroprotection and seroconversion rates and HAI titer GMFIs at day 28 were similar in participants with AD who received intradermal or intramuscular vaccination for each of the 3 strains (Table II).

TABLE I. Demographic and baseline characteristics

Characteristic	NA ID (n = 111)	Moderate/severe AD ID (n = 100)	Moderate/severe AD IM (n = 102)	NA IM (n = 23)
Sex, no. (%)				
Female	66 (59.5)	56 (56.0)	57 (55.9)	12 (52.2)
Male	45 (40.5)	44 (44.0)	45 (44.1)	11 (47.8)
Race, no. (%)				
Black or African American	21 (18.9)	30 (30.0)	41 (40.2)	3 (13.0)
White	78 (70.3)	55 (55.0)	52 (51.0)	16 (69.6)
Other	12 (10.8)	15 (15.0)	9 (8.8)	4 (17.4)
Ethnicity, no. (%)				
Hispanic or Latino	10 (9.0)	9 (9.0)	10 (9.8)	7 (30.4)
Not Hispanic or Latino	101 (91.0)	91 (91.0)	92 (90.2)	16 (69.6)
Age (y), mean (SD)	38.8 (11.9)*	35.4 (11.3)	36.6 (12.1)	34.3 (9.8)
Total IgE (kU/L), median (Q1-Q3)	24.2 (10.1-65.3)*	196.5 (41.6-1168.5)	294.0 (85.9-1017.0)	24.9 (8.9-79.8)
Eosinophils (cells/μL), median (Q1-Q3)	0.10 (0.05-0.16)*	0.20 (0.12-0.35)	0.19 (0.09-0.38)	0.10 (0.07-0.13)
EASI score, median (Q1-Q3)	Not applicable	9.80 (4.1-18.8)	9.03 (4.2-22.3)	Not applicable
Rajka-Langeland Total Score, median (Q1-Q3)	Not applicable	7 (6-8)	7 (6-8)	Not applicable
Rajka-Langeland severity categories, no. (%)				
Moderate (4.5-7.5)	Not applicable	67 (67.0)	69 (67.6)	Not applicable
Severe (8-9)	Not applicable	33 (33.0)	33 (32.4)	Not applicable
S aureus skin colonization, no. (%)				
Positive	1 (0.9)*	38 (38.0)	46 (45.1)	3 (13.0)
Negative	110 (99.1)	60 (60.0)	56 (54.9)	20 (87.0)
Missing	0 (0.0)	2 (2.0)	0 (0.0)	0 (0.0)

Percentages are based on column totals (numbers). For total IgE, 1 kU/L = 2.4 μg/L.

EASI, Eczema Area and Severity Index; *ID*, intradermal; *IM*, intramuscular; *NA*, nonatopic; *Q1*, first quartile; *Q3*, third quartile.

*Differences between the nonatopic intradermal group and the moderate/severe AD intradermal group ($P < .05$). Pairwise comparisons are based on the Fisher exact test for proportions and the Wilcoxon 2-sample test for continuous measures. There were no differences between the moderate/severe AD intradermal group and the moderate/severe AD intramuscular group for any measure. No other pairwise comparisons were assessed.

Comparative antibody responses of nonatopic participants after intradermal and intramuscular vaccination

As an exploratory analysis, the seroprotection rates of nonatopic participants were similar between those given intradermal vaccination and those given intramuscular vaccination (Table II).

Effect of *S aureus* skin colonization on antibody responses to vaccination

Results of *S aureus* cultures of skin swabs were available in 334 (99%) of 336 participants; cultures were collected up to 477 days before vaccination (mean, 143 days) in 330 (99%) of 334 participants and after vaccination (mean, 37 days) in 4 (1%) of 334 participants. Cultures for 120 (36%) participants were collected within 30 days of vaccination, with cultures for 70 participants collected the same day as vaccination. Overall, 42% of participants with AD were colonized (Table I). Among all participants with AD who were not seroprotected at baseline separately for influenza B, H1N1, and H3N2, the rates of SASC were 41%, 44%, and 41%, respectively. Participants with AD and SASC were divided evenly between intradermal and intramuscular vaccines.

Baseline TSST-1–specific and SEB-specific IgE and IgG antibodies were higher in participants with AD with SASC compared with participants with AD without SASC (see Fig E2 in this article's Online Repository at www.jacionline.org). Also, participants with AD without SASC had higher baseline TSST-1 and SEB antibody levels than nonatopic participants without SASC.

Comparative antibody responses to intradermal vaccination

The rate of seroprotection to influenza B in participants with AD with SASC was lower than in participants with AD without SASC (11% vs 47%; OR, 0.14; 95% CI, 0.03-0.49; $P < .001$; Fig 2). The difference in the rate of seroprotection to influenza B persisted when including only participants with moderate AD (6% vs 51%; OR, 0.06; 95% CI, 0.00-0.50; $P = .002$) or when including only participants with *S aureus* cultures of skin swabs collected within 30 days of vaccination (0% vs 57%; OR, 0.00; 95% CI, 0.00-0.40; $P = .004$).

Additionally, there was a trend among participants with AD toward a lower H1N1 strain seroprotection rate in those with SASC compared with those without (74% vs 91%; OR, 0.27; 95% CI, 0.04-1.37; $P = .09$).

Among participants with AD, the rate of seroconversion to influenza B and the rate of seroconversion to H1N1 in participants with SASC were also lower than those without SASC (19% vs 52%; OR, 0.23; 95% CI, 0.07-0.64; $P = .002$ and 74% vs 94%; OR, 0.17; 95% CI, 0.02-1.06; $P = .03$, respectively; Fig 2), which persisted for influenza B when including only participants with moderate AD (25% vs 55%; OR, 0.27; 95% CI, 0.06-1.08; $P = .05$) or when including only participants with skin swabs collected within 30 days of vaccination (11% vs 65%; OR, 0.07; 95% CI, 0.00-0.70; $P = .02$).

Participants with AD and SASC had lower HAI titer GMFIs against influenza B compared with participants with AD without SASC (GMR, 0.50; 95% CI, 0.34-0.74; $P < .001$; see Fig E3 in this article's Online Repository at www.jacionline.org), which persisted when including only participants with AD (GMR, 0.52; 95% CI, 0.31-0.89; $P = .02$) or when including only

TABLE II. Baseline and day 28 postvaccination immune response summary

	NA ID	Moderate/ severe AD ID vs NA ID	Moderate/ severe AD ID	Moderate/ severe AD ID vs AD IM	Moderate/ severe AD IM	NA IM
B						
No.	102		96		92	18
Baseline GMT (95% CI)	7.5 (6.8-8.3)		6.7 (6.1-7.3)		7.4 (6.7-8.2)	6.1 (4.8-7.6)
Day 28 GMT (95% CI)	16.4 (13.6-19.8)		20.4 (16.3-25.6)		21.4 (17.5-26.2)	20.0 (15.0-26.7)
GM fold increase (95% CI)	2.2 (1.8-2.6)		3.1 (2.4-3.9)		2.9 (2.4-3.5)	3.3 (2.2-4.9)
GM ratio (95% CI)		1.25 (0.96-1.61)		0.93 (0.71-1.22)		
		$P = .09$		$P = .61$		
Seroprotection (% [95% CI])	23% (15% to 32%)		34% (25% to 45%)		34% (24% to 44%)	22% (6.4% to 48%)
Odds ratio (95% CI)		1.80 (0.92-3.55)		1.03 (0.54-1.97)		
		$P = .08$		$P > .99$		
Seroconversion % [95% CI])	30% (22% to 40%)		41% (31% to 51%)		48% (37% to 58%)	61% (36% to 83%)
Odds ratio (95% CI)		1.57 (0.84-2.94)		0.75 (0.40-1.38)		
		$P = .14$		$P = .38$		
H1N1						
No.	59		63		53	10
Baseline GMT (95% CI)	7.9 (6.8-9.1)		7.8 (6.7-9.0)		8.8 (7.5-10.3)	12.3 (7.7-19.7)
Day 28 GMT (95% CI)	230 (151-352)		142 (95.6-210)		180 (125-260)	106 (43.7-255)
GM fold increase (95% CI)	29.1 (18.3-46.3)		18.3 (12.3-27.1)		20.5 (13.4-31.3)	8.6 (3.0-24.7)
GM ratio (95% CI)		0.59 (0.32-1.11)		0.91 (0.50-1.65)		
		$P = .10$		$P = .75$		
Seroprotection (% [95% CI])	86% (75% to 94%)		84% (73% to 92%)		92% (82% to 98%)	90% (55% to 100%)
Odds ratio (95% CI)		0.83 (0.26-2.56)		0.43 (0.09-1.63)		
		$P = .80$		$P = .25$		
Seroconversion (% [95% CI])	88% (77% to 95%)		86% (75% to 93%)		89% (77% to 96%)	60% (26% to 88%)
Odds ratio (95% CI)		0.81 (0.24-2.65)		0.77 (0.21-2.62)		
		$P = .79$		$P = .78$		
H3N2						
No.	60		59		49	11
Baseline GMT (95% CI)	8.8 (7.6-10.2)		9.8 (8.3-11.5)		9.1 (7.7-10.7)	8.3 (5.7-11.9)
Day 28 GMT (95% CI)	79.1 (54.4-115)		93.2 (64.1-135)		108 (78.2-148)	75.1 (29.3-193)
GM fold increase (95% CI)	9.0 (6.2-13.0)		9.5 (6.6-13.9)		11.9 (8.2-17.2)	9.1 (3.2-25.6)
GM ratio (95% CI)		1.04 (0.60-1.80)		0.76 (0.44-1.31)		
		$P = .90$		$P = .32$		
Seroprotection (% [95% CI])	73% (60% to 84%)		85% (73% to 93%)		94% (83% to 99%)	91% (59% to 100%)
Odds ratio (95% CI)		2.02 (0.75-5.71)		0.36 (0.06-1.58)		
		$P = .18$		$P = .22$		
Seroconversion (% [95% CI])	73% (60% to 84%)		76% (63% to 86%)		88% (75% to 95%)	82% (48% to 98%)
Odds ratio (95% CI)		1.17 (0.47-2.92)		0.45 (0.13-1.39)		
		$P = .83$		$P = .14$		

HAI antibody titers of less than 1:10 have been imputed as 1:5, and titers of 1:1280 or greater have been imputed as 1:2560 for analyses. Geometric mean titer and geometric mean fold increase statistics are raw estimates. Geometric mean ratios, 95% CIs, and *P* values are from pairwise robust regression models of $\log_2$ HAI titer fold increase, adjusting for age and sex. ORs and 95% CIs are exact estimates, and *P* values are from pairwise Fisher exact tests. Geometric mean ratios and ORs compare the moderate/severe AD intradermal group with the adjacent groups.

GMT, Geometric mean titer; *GM*, geometric mean; *ID*, intradermal; *IM*, intramuscular; *NA*, nonatopic.

participants with skin swabs collected within 30 days of vaccination (GMR, 0.39; 95% CI, 0.17-0.88; $P = .02$).

Although the HAI titer GMFI against influenza B was influenced by the presence of SASC, it was not influenced by the level of AD severity among intradermal vaccinees. When considering both SASC status and AD severity as covariates in a robust regression model including intradermally vaccinated participants with AD, there were no pairwise differences in HAI titer GMFIs against influenza B between severity levels and only marginal evidence of an overall effect of severity ($P = .07$). However, there was evidence of an overall effect of SASC status on HAI titer GMFIs against influenza B ($P = .01$).

In *post hoc* analyses, among participants without SASC receiving intradermal vaccination, the seroprotection rates were lower in nonatopic participants than in participants with AD for influenza B (23% vs 47%; OR, 0.34; 95% CI, 0.16-0.72; $P = .003$), and the seroconversion rate for the B strain was also lower in nonatopic participants than in participants with AD (31% vs 52%; OR, 0.41; 95% CI, 0.20-0.85; $P = .01$; Fig 2).

Comparative antibody responses to intramuscular vaccination

Among participants with AD receiving intramuscular vaccination, SASC status did not affect either the seroprotection rate or the seroconversion rate to any of the 3 strains (Fig 2). There was a trend, among participants with AD vaccinated intramuscularly, toward a lower HAI titer GMFI against influenza H3N2 in

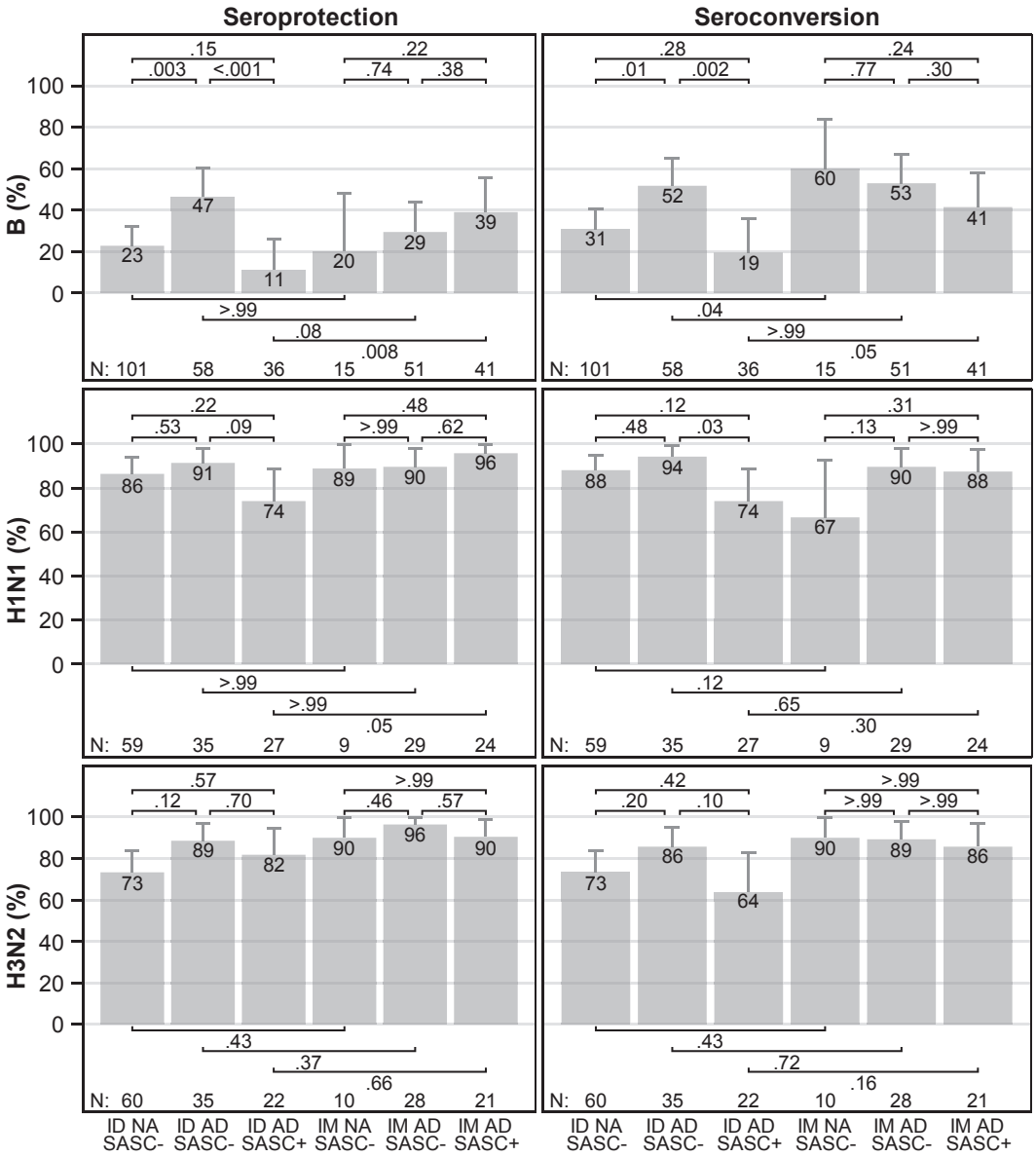


FIG 2. Day 28 postvaccination influenza B, H1N1, and H3N2 seroprotection and seroconversion by vaccination route, diagnostic group, and *S aureus* skin colonization. Seroprotection (HAI titers ≥ 1:40) and seroconversion (4-fold or greater increase in HAI titers over baseline titers) percentages and upper 95% CIs are displayed. Pairwise comparisons are performed by using the Fisher exact test. *ID*, Intradermal; *IM*, intramuscular; *NA*, nonatopic.

participants with SASC than in participants without SASC (GMR, 0.49; 95% CI, 0.23-1.08; $P = .08$; see Fig E3).

Comparison of antibody responses between intradermal and intramuscular vaccination in participants with AD and SASC

In a *post hoc* analysis the proportion of participants with AD with SASC achieving seroprotection to influenza B was lower among those receiving intradermal vaccination than among those receiving intramuscular vaccination (11% vs 39%; OR, 0.20; 95% CI, 0.04-0.72; $P = .008$; Fig 2). There were similar trends in seroconversion to influenza B (19% vs 41%; OR, 0.34; 95% CI, 0.10-1.06; $P = .05$) and in seroprotection to influenza H1N1 (74% vs 96%; OR, 0.12; 95% CI, 0.00-1.14; $P = .05$; Fig 2). Vaccination route did not influence immune responses to influenza H3N2 among participants with AD with SASC.

Intradermal vaccination resulted in a lower HAI titer GMFI to the B strain than intramuscular vaccination in participants with AD with SASC (GMR, 0.64; 95% CI, 0.45-0.90; $P = .01$; see Fig E3).

Comparison of antibody responses between intradermal and intramuscular vaccination in participants with AD and without SASC

In a *post hoc* analysis there were no differences between responses to intradermal and intramuscular vaccinations among participants with AD without SASC (Fig 2 and see Fig E3).

Effect of *S aureus* skin colonization status on influenza B–specific IgG_1, IgG_2, IgG_3, and IgA responses to intradermal vaccination among participants with AD

There were no differences in baseline IgG_1, IgG_2, IgG_3, or IgA titers to influenza B between participants with AD with and without SASC (data not shown). Participants with AD and SASC had lower day 28 IgG_1 responses to influenza B than participants with AD without SASC (GMR, 0.82; 95% CI, 0.69-0.97; $P = .02$), whereas there were no such differences in day 28 IgG_2, IgG_3, or IgA responses to influenza B (see Figs E4 and E5 in this article's Online Repository at www.jacionline.org). There were no differences in day 28 IgG_1, IgG_2, IgG_3, or IgA responses to influenza B between intradermal and intramuscular vaccination among participants with AD with SASC.

Comparison of antibody response by sex and race

There were no differences in seroconversion, seroprotection, or HAI titer GMFIs to any vaccine strain between male and female participants or between white and black or African American participants within the groups of participants with AD or nonatopic participants, regardless of vaccination route (data not shown).

Safety summary

A total of 4 adverse events, 2 nonserious and 2 serious and requiring hospitalization, were reported among 3 subjects. All adverse events were grade 3, resolved without sequelae, and deemed not related to the vaccination. One participant with AD receiving intramuscular vaccination experienced simultaneous vomiting and diarrhea 6 days after vaccination, 1 participant with AD receiving intradermal vaccination was hospitalized for an asthma exacerbation 6 days after vaccination, and 1 nonatopic participant receiving intradermal vaccination was hospitalized for a skin infection.

DISCUSSION

The current study is the first immunologic examination of intradermal vaccination against influenza in patients with AD. Seroprotection and seroconversion rates were not different overall between participants with AD and nonatopic control subjects receiving intradermal vaccination for any of the 3 influenza strains (B, H1N1, and H3N2). In contrast, after intradermal vaccination in participants with AD, compared with those without SASC, participants with SASC experienced (1) lower seroprotection and seroconversion rates and lower HAI titer GMFIs against influenza B and (2) lower seroconversion rates against influenza H1N1. However, among participants with AD with SASC, the response rate is higher among those receiving intramuscular vaccination than those receiving intradermal vaccination.

Most differences were seen in response to influenza B. This result is probably due to a new B strain in the vaccine and low immunogenicity of the B strain.[16,17] The low immunogenicity of the B strain is an important handicap of inactivated influenza vaccines because recent studies show that the B strain is not less pathogenic than the A strain.[18]

The antibody response to influenza vaccines is mainly found within the IgG_1 antibody subclass.[19] Therefore we analyzed IgG_1, IgG_2, IgG_3, and IgA antibody responses to influenza. Our finding that IgG_1 antibody responses after intradermal vaccination were reduced in participants with AD with SASC provided further support for a deficient cutaneous vaccination response in participants with AD with SASC (see Fig E4).

It is not known whether SASC is simply a biomarker for reduced immune responses to intradermal vaccination or whether *S aureus* directly inhibits immune responses to intradermal vaccination in participants with AD. We considered the possibility that this association of diminished intradermal vaccine response because of *S aureus* colonization was related to AD severity. However, when we controlled for the severity of skin disease, SASC remained strongly associated with reduced intradermal vaccine response to influenza vaccination. Previous studies have demonstrated that staphylococcal superantigenic toxins deplete dendritic cells from the skin by inducing migration of cutaneous antigen-presenting cells to the draining lymph nodes.[20] Furthermore, it is known that *S aureus* products, such as staphylococcal protein A, have subversive effects on B-cell and plasmablast antibody responses.[21] This provides biologic plausibility for the association of *S aureus* colonization with reduced vaccine antibody responses.

Previous ADRN studies of transcutaneous vaccination to yellow fever virus in skin of patients with AD revealed an inverse association between total serum IgE levels and neutralizing antiviral antibody titers.[22] In the current study of intradermal vaccination, however, reduced anti-influenza antibody responses were independent of baseline serum IgE levels. Our data suggest that the immunologic characteristics of the skin compartment and microbiome might dictate immune responses to influenza vaccines in patients with AD.

Considering that AD is a common health problem, these patients, as well as those with other skin diseases, should be evaluated during early-stage clinical trials that involve cutaneous delivery. New biomarkers, such as total serum IgE and SASC, might prove useful to identify population subsets that might not respond optimally to intradermal vaccination.

A limitation of our current study is that skin swabs for *S aureus* were not collected on the day of vaccination for 79% of participants. However, microbiologic studies have demonstrated that *S aureus* colonization can affect more than 90% of patients with severe AD.[23] Persistent *S aureus* colonization in participants with AD for up to 1 year has been demonstrated in other studies, suggesting that skin swabs obtained at different time points will be relevant to future propensity to *S aureus* colonization.[24-26] Another limitation of our study is that because intradermal vaccination is only approved for adults, the current study did not include children. However, it is immunologically plausible that *S aureus* colonization subverts the skin immune response because eczema herpeticum in all age groups is associated with *S aureus* colonization.[27]

In our current study we conclude that nonatopic participants and participants with AD overall mount similar immune

responses to intradermal vaccination. However, the subset of participants with AD with SASC exhibited reduced immune responses after intradermal vaccination compared with participants with AD without SASC. Patients with AD without *S aureus* colonization had stronger seroprotection and seroconversion against influenza B than nonatopic control subjects ($P = .003$ and $P = .01$, respectively) and *S aureus*–colonized patients with AD ($P < .001$ and $P = .002$, respectively) when they were vaccinated intradermally, suggesting the local environment of the *S aureus*–colonized skin subverts vaccine immune responses. Because SASC has been reported in the majority of AD,[23] the most prudent approach will be to avoid intradermal influenza vaccination in patients with AD when a suitable vaccine with an alternative route of administration is available and not contraindicated.

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Clinical implications: Patients with AD colonized with *S aureus* exhibit reduced immune responses to influenza vaccination compared with noncolonized patients after intradermal but not intramuscular vaccination. Intramuscular influenza vaccination should be given preference in *S aureus*–colonized patients.

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METHODS

Oral and written informed consent were obtained from the study participants. Each participant received a stipend for participating in this protocol.

Ethnicity and race derivation

Ethnicity categories were self-reported from the options of (1) Hispanic or Latino and (2) not Hispanic or Latino. Race categories are reported as follows: (1) black or African American, (2) white, and (3) other. Participants self-reported their own race or races from a list of prespecified case report form race categories, from which general race categories were derived (case report form categories and corresponding derived race categories are defined in Table E1). More than 1 self-reported option could be selected. Race was assessed to investigate differences in immune response between groups.

Eligibility criteria

Inclusion criteria included the following:

- Male and female subjects 18 to 64 years of age, inclusive, on the day of vaccination.
- Enrolled in the ADRN Registry study. (https://www.clinicaltrials.gov/ct2/show/NCT01494142?term=adrn&rank=4).
- Had active mild-to-severe AD (lesions present) with or without a history of eczema herpeticum or were nonatopic, as diagnosed by using the ADRN Standard Diagnostic Criteria
- Willing to sign the informed consent form before initiation of any study procedure.

Exclusion criteria included the following:

- Were pregnant or lactating. Women of child-bearing potential were to avoid becoming pregnant (use of an effective method of contraception or abstinence) for the duration of their participation in the study.
- Had a known allergy to any component of the Fluzone Intradermal or Fluzone (Intramuscular) vaccines, including egg protein, or had a severe allergic reaction to a previous dose of any influenza vaccine.
- Had a known or suspected congenital or acquired immunodeficiency or who had immunosuppressive therapy (excluding steroids), such as anticancer chemotherapy or radiation therapy within 4 weeks before the day of vaccination.
- Received systemic steroid therapy for 2 or more weeks at a dose of 20 mg/d or greater prednisone equivalent within 4 weeks before the day of vaccination or expected to receive within 3 weeks after vaccination.
- Received a cumulative dose of inhaled and/or intranasally administered corticosteroids of 880 μg/d or greater fluticasone equivalent for 2 or more weeks within 4 weeks before the day of vaccination or expected to receive within 3 weeks after vaccination.
- Had a chronic illness, including but not limited to cardiac, renal, or autoimmune disorders, or diabetes at a stage that could interfere with study conduct or completion, based on the opinion of the investigator. Asthma and underlying allergic conditions, such as allergic rhinitis, were not exclusionary.
- Had a neoplastic disease or any hematologic malignancy; uncomplicated nonmelanoma skin cancer and melanoma *in situ* with documentation of complete excision were not exclusionary. Participants who were disease free for at least 6 months were not excluded.
- Participated in another clinical trial investigating a vaccine, drug, medical device, or a medical procedure in the 4 weeks preceding the study vaccination or who planned to participate in another clinical trial during the study period.
- Had any skin disease other than AD that might compromise the stratum corneum barrier (eg, bullous disease, psoriasis, cutaneous T-cell lymphoma [also called mycosis fungoides or Sezary syndrome], dermatitis herpetiformis, Hailey-Hailey, or Darier disease).
- Received blood or blood-derived products that might interfere with the assessment of immune response in the past 3 months before vaccination or who planned to receive such products during the study period.
- Received previous vaccination (Fluzone or another vaccine) against influenza in the past 6 months before vaccination.
- Received any other live vaccines within 4 weeks or inactivated vaccines within 2 weeks before study vaccination or who planned to receive any vaccination during the study period.
- Had thrombocytopenia or bleeding disorder in the 3 weeks preceding vaccination.
- Had a personal or family history of Guillain-Barré syndrome.
- Had a first-degree relative already enrolled in the study.
- Determined to be ineligible based on the opinion of the investigator.
- Received phototherapy (eg, UVB, psoralen plus UVA, or tanning bed) within the last 5 days before vaccination.

Temporary exclusion criteria included the following:

- Signs and symptoms of an acute infectious respiratory illness.
- Febrile illness (temperature ≥37.5°C [or ≥99.5°F]) or moderate or severe acute illness/infection on the day of vaccination.
- AD flare, a worsening of the AD participant's skin condition requiring increased level of baseline treatment during the previous seven days. Participant must not have applied topical corticosteroids or calcineurin inhibitors to the deltoid region of the extremity to be vaccinated in the 7 days before vaccination.
- Had taken nonsteoridal anti-inflammatory drugs or acetaminophen within 24 hours before the time of vaccination.

Methodology of *S aureus* culture and laboratory assays

***S aureus* culture.** Skin swabs were obtained as part of the ADRN Registry protocol to conduct *S aureus* cultures. For patients with AD, lesional (most severe lesion) and nonlesional swabs were collected. Nonlesional swabs were collected for nonatopic subjects. The body site priorities for swabbing were the (1) extremities, (2) face/neck, and (3) trunk. Participants had not taken oral antibiotics or topical prescription medications within 7 days before swabbing and had not taken systemic immunosuppressive drugs within 20 days before swabbing. In brief, a skin swab (BD BBL CultureSwab, Liquid Stuart's Transport, and Single Swab) was moistened with nonbacteriostatic saline and used to swab a 5 × 5–cm area. Swabs were then used to inoculate blood agar plates (5% sheep blood; no. R01202; Remel, Lenexa, Kansas). Plates were incubated up to 48 hours in a 5% CO_2 incubator at 37°C. Colonies appearing to be *S aureus* were tested for coagulase and catalase. If the test results were positive, the colonies were identified as *S aureus* species. SASC was defined based on *S aureus* growth in skin cultures. Participants with AD were defined as having SASC if either lesional or nonlesional skin showed *S aureus* growth.

IgE and IgG antibodies specific for SEB or TSST-1. IgE and IgG antibodies specific for SEB or TSST-1 were measured by using a fluoroenzyme immunoassay (FEIA; ImmunoCAP 250; Thermo Fisher Scientific, Kalamazoo, Mich). The ImmunoCAP is a computer-driven autoanalyzer that uses a solid-phase immunometric (labeled antibody) assay chemistry.[E1-E3]

HAI antibody titers. Assays were performed, as previously described,[E4] using antigens from the Investigator Reagent Resource, Centers of Disease Control. Sera were incubated at 1:4 in receptor-destroying enzyme solution (Denka Seiken, Tokyo, Japan) for 30 minutes at 56°C and subsequently with turkey red blood cells at 4°C for 60 minutes to remove nonspecific hemagglutinins. Serial 2-fold dilutions in PBS starting at 1:10 were mixed with 4 HA units of each vaccine virus antigen and turkey red blood cells in 96-well V-bottom microtiter plates (Corning, Corning, NY) for 30 minutes at room temperature. The HAI titer was defined as the reciprocal of the last serum dilution with no HAI activity. A titer of 5 was assigned to samples in which the first dilution was negative. Each run included high- and low-titer positive controls. Assays were considered valid if the control HAI titers had 2-fold or less differences from their previously established mean titers.

Influenza B strain–specific IgG_1, IgG_2, IgG_3, and IgA concentrations. Influenza B strain–specific IgG1, IgG2, and IgG3

antibody levels were measured by means of capture ELISA with precoated 96-well microtiter plates (M1551; Cell Sciences, Newburyport, Mass). Plates were incubated at 37°C for 1.5 hours with the test sera diluted at 1:2 for IgG1, undiluted for IgG2, and diluted 1:4 for IgG3 in the kit-provided diluent. For IgG3 plates, wells were first blocked with 20% FBS for 1 hour and washed before incubating with sera. After washing, 8 HA units of B/Wisconsin/1/2010 in 100 μL of PBS were added to each well and incubated overnight at 4°C. Wells were washed the following day, and 100 μL of goat anti–influenza B (AB1058; Millipore, Temecula, Calif) diluted 1:100 in kit diluent were added to each well. After 1 hour at room temperature, wells were washed, and 100 μL of anti-goat–horseradish peroxidase (6300-05; SouthernBiotech, Birmingham, Ala) diluted 1:1000 in diluent were added to each well. After 1 hour at room temperature, wells were washed, and bound antibodies were revealed with ABTS (0401-01; SouthernBiotech). Influenza B strain–specific IgA antibodies were measured by coating 96-well plates with IgA antibody at 2 μg/mL (3860-1AD; Mabtech, Cincinnati, Ohio) overnight. Wells were washed and incubated for 2 hours with test sera diluted 1:20 in diluent. After washing, 8 HA units of B/Florida/4/2006 in 100 μl of PBS was added to each well and incubated at 4°C overnight. Wells were washed and 100 μL of anti-influenza B–horseradish peroxidase (ab20039; Abcam, Cambridge, United Kingdom) were added to each well. After 1 hour at room temperature, wells were washed, and bound antibodies were revealed with ABTS (0401-01; SouthernBiotech). ODs were read with a 405-nm filter on a Multiskan FC instrument (Thermo Fisher). Antibody concentrations were interpolated against the kit-supplied standard curve by using a sigmoidal dose-response variable slope analysis and Prism5 software (GraphPad Software, La Jolla, Calif).

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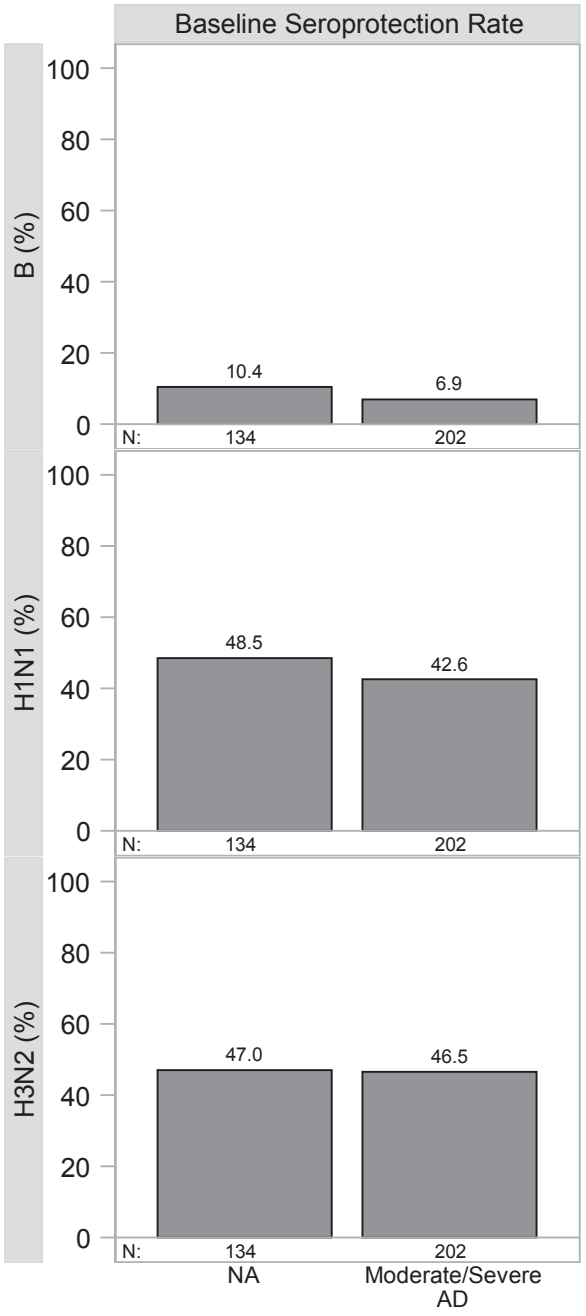

FIG E1. Baseline influenza B, H1N1, and H3N2 seroprotection by diagnostic group. Baseline seroprotection is an HAI titer of 1:40 or greater before vaccination. *NA*, Nonatopic.

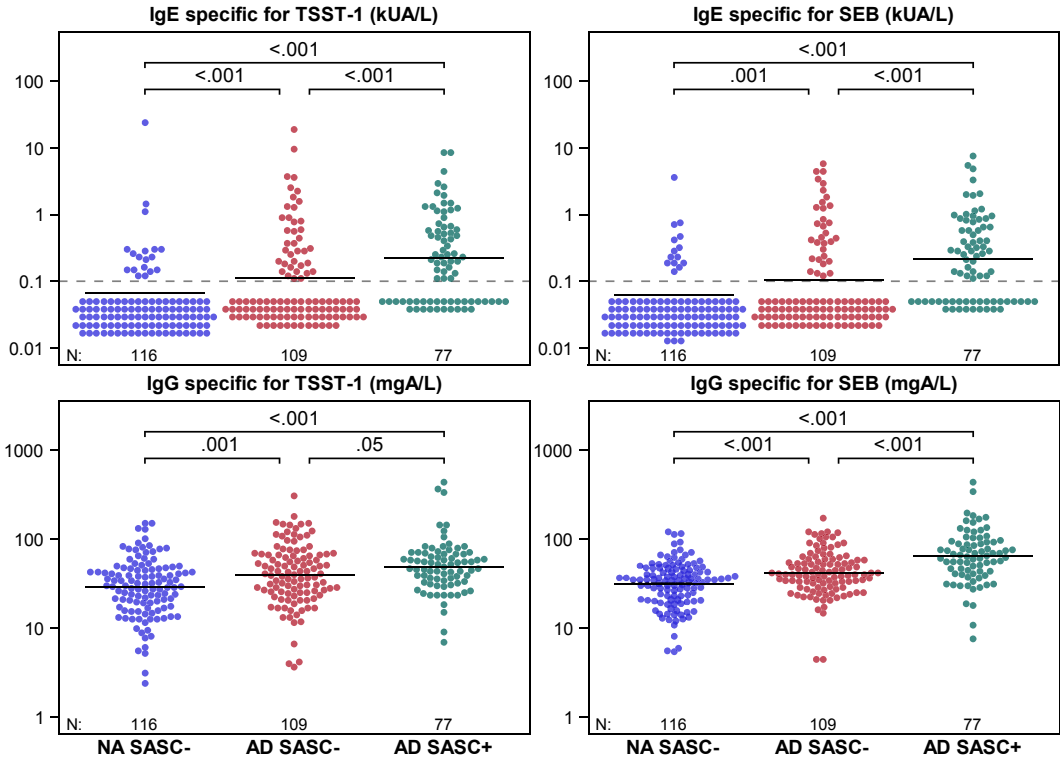

FIG E2. Baseline IgE and IgG specific for SEB and TSST-1 by diagnostic group and *S aureus* skin colonization. Unadjusted geometric means are displayed for each group. Pairwise comparisons are based on left-censored regression models on the $\log_{10}$ scale. Values of less than the lower limit of detection (0.1) are plotted on the bottom of the panel, if applicable. For IgE, 1 kUA/L = 2.4 μg/L; for IgG, 1 mgA/L = 1 mg/L. *NA*, Nonatopic.

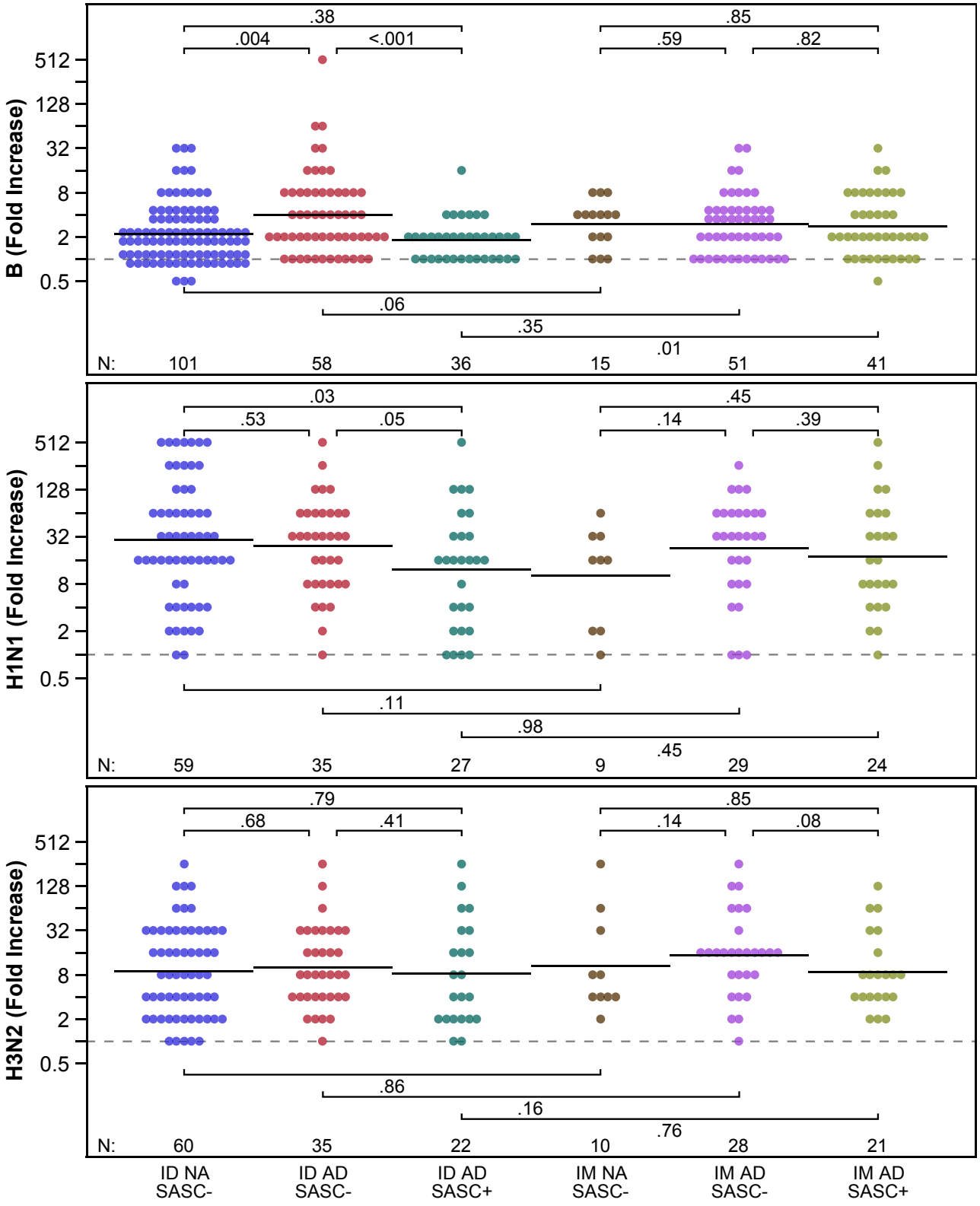

FIG E3. Day 28 postvaccination influenza B, H1N1, and H3N2 HAI titer fold increase over baseline by vaccination route, diagnostic group, and *S aureus* skin colonization status. Unadjusted geometric means are displayed for each group. Pairwise comparisons are based on robust regression models of the $\log_2$ HAI titer fold increase. HAI antibody titers of less than 1:10 have been imputed as 1:5, and titers of 1:1280 or greater have been imputed as 1:2560 for analyses prior to calculating the fold increase. *ID*, Intradermal; *IM*, intramuscular; *NA*, nonatopic.

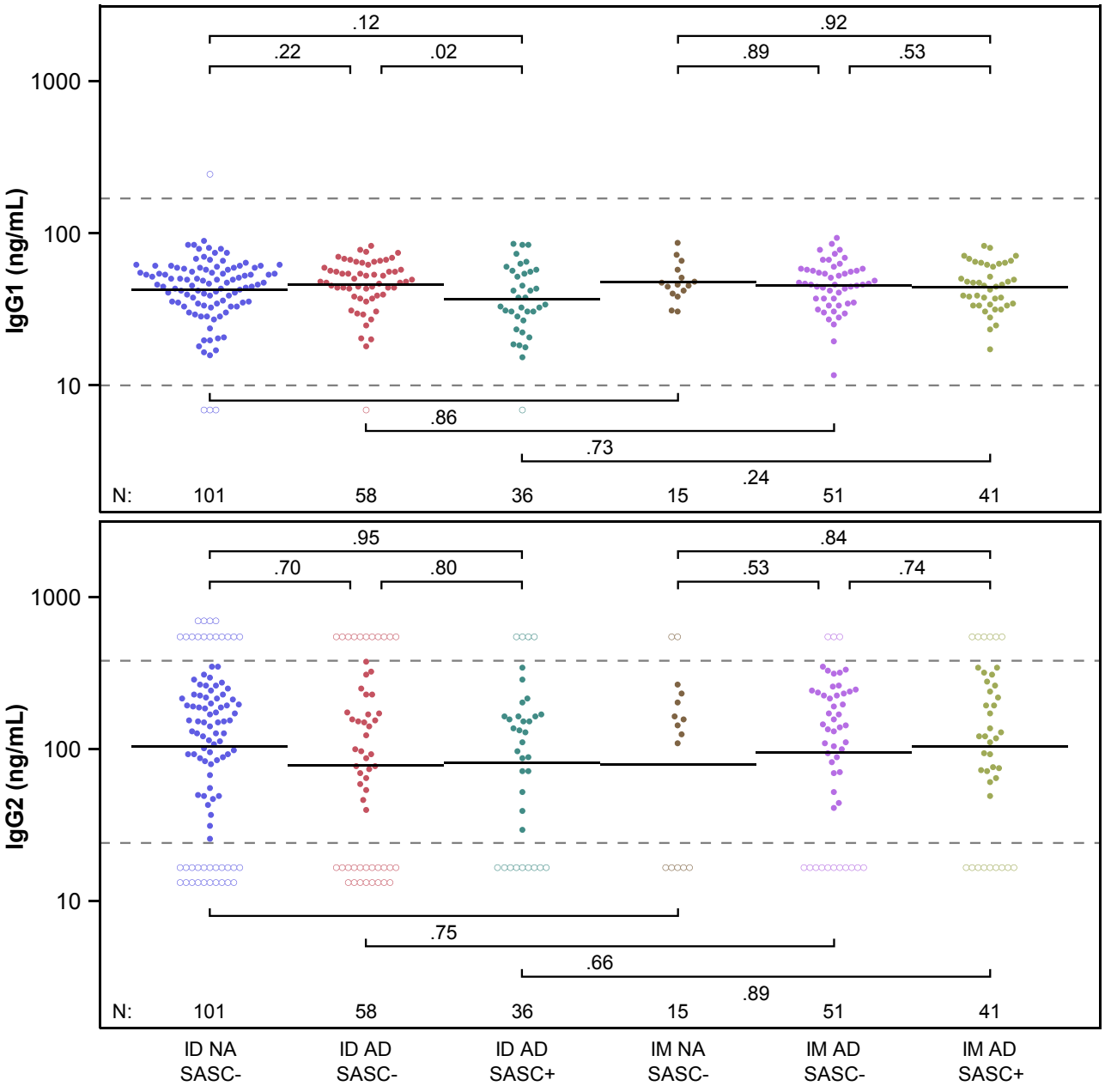

FIG E4. Day 28 postvaccination influenza B–specific IgG_1 and IgG_2 levels by vaccination route, diagnostic group, and *S aureus* skin colonization. Unadjusted geometric means are displayed for each group. Pairwise comparisons are based on robust regression models of the log_{10} value. Values outside the limits of quantification (IgG_1, 10 and 170; IgG_2, 24 and 380 for lower and upper limits, respectively) are imputed by using multiple imputation methods and are indicated at the top and bottom of the plots. 10,000 ng/mL = 1 mg/dL. *ID*, Intradermal; *IM*, intramuscular; *NA*, nonatopic.

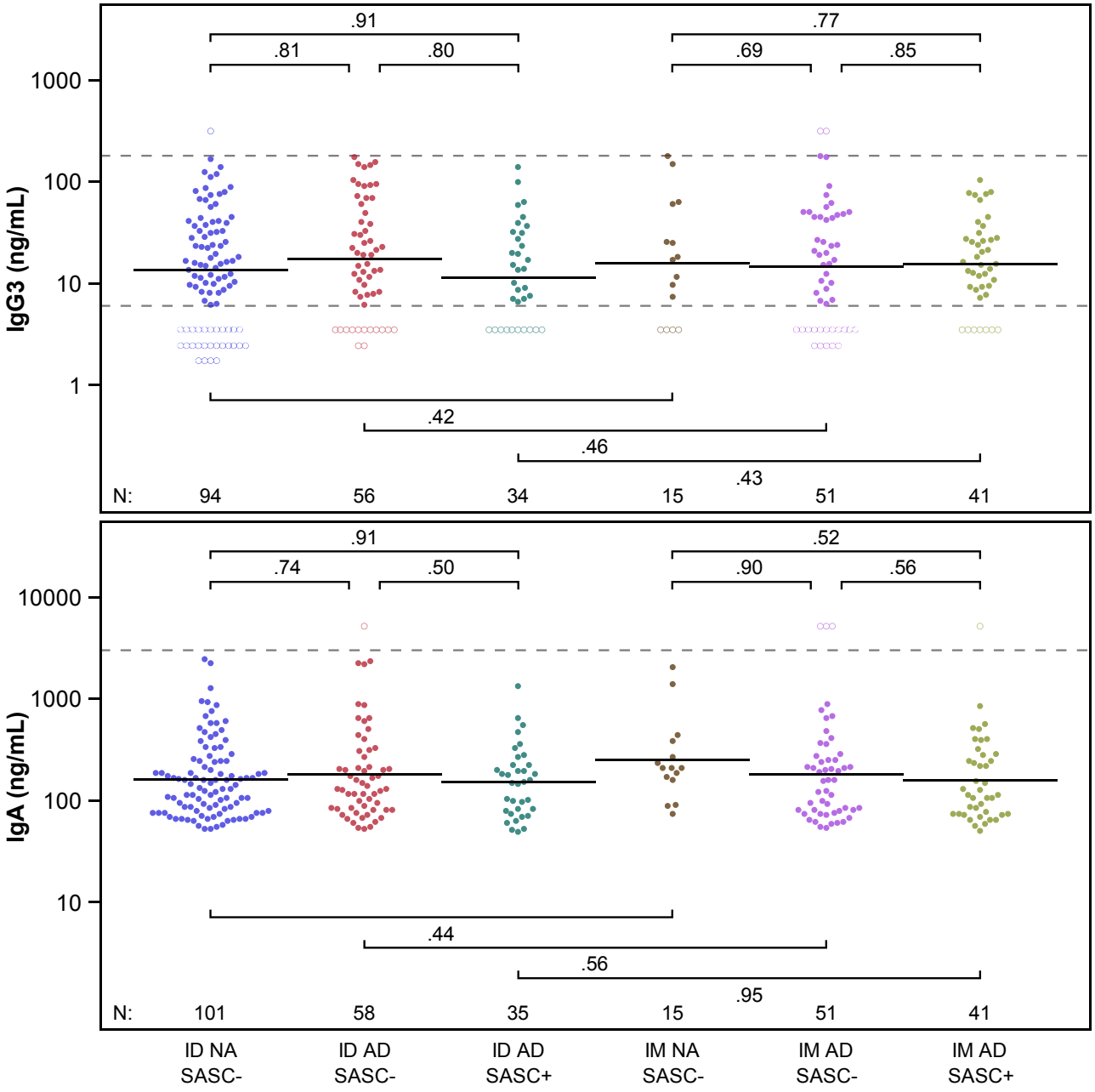

FIG E5. Day 28 postvaccination influenza B–specific IgG_3 and IgA levels by vaccination route, diagnostic group, and *S aureus* skin colonization. Unadjusted geometric means are displayed for each group. Pairwise comparisons are based on robust regression models of the log_{10} value. Values outside the limits of quantification (IgG_3, 6 and 180 for lower and upper limits, respectively; IgA, 3000 for upper limit) are imputed by using multiple imputation methods and are indicated at the top and bottom of the plots. 10,000 ng/mL = 1 mg/dL. *ID*, Intradermal; *IM*, intramuscular; *NA*, nonatopic.

TABLE E1. Race classification

Case report form race option	Derived race category
Black or African American (check all that apply) African American Caribbean/West Indian African	Black or African American
White	White
American Indian or Alaska Native	Other
Asian Indian	
Filipino	
Chinese	
Japanese	
Korean	
Vietnamese	
Native Hawaiian	
Guamanian or Chamorro	
Samoan	
Other Pacific Islander	
Other East Asian	
Other West Asian	
Other	
More than 1 option selected (unless each option fits the Black or African American category)	