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Immune Responses in U.S. Military Personnel Who Received Meningococcal Conjugate Vaccine (MenACWY) Concomitantly with Other Vaccines Were Higher than in Personnel Who Received MenACWY Alone

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Immunological responses to vaccination can differ depending on whether the vaccine is given alone or with other vaccines. This study was a retrospective evaluation of the immunogenicity of a tetravalent meningococcal conjugate vaccine for serogroups A, C, W, and Y (MenACWY) administered alone ($n = 41$) or concomitantly with other vaccines ($n = 279$) to U.S. military personnel (mean age, 21.6 years) entering the military between 2006 and 2008. Concomitant vaccines included tetanus/diphtheria (Td), inactivated polio vaccine (IPV), hepatitis vaccines, and various influenza vaccines, among others; two vaccine groups excluded Tdap and IPV. Immune responses were evaluated in baseline and postvaccination sera for *Neisseria meningitidis* serogroups C and Y 1 to 12 months (mean, 4.96 months) following vaccination. Functional antibodies were measured by using a serum bactericidal antibody assay with rabbit complement (rSBA) and by measurement of serogroup-specific immunoglobulin G (IgG) antibodies. The percentage of vaccinees reaching threshold levels (IgG concentration in serum, ≥2 μg/ml; rSBA titer, ≥8) corresponding to an immunologic response was higher postvaccination than at baseline ($P < 0.001$). Administration of MenACWY along with other vaccines was associated with higher geometric means of IgG concentrations and rSBA titers than those measured 4.60 months after a single dose of MenACWY. In addition, higher percentages of vaccinees reached the immunological threshold (range of odds ratios [ORs], 1.5 to 21.7) and more of them seroconverted (OR range, 1.8 to 4.8) when MenACWY was administered with any other vaccine than when administered alone. Additional prospective randomized clinical trials are needed to confirm the observed differences among groups in the immune response to MenACWY when given concomitantly with other vaccines to U.S. military personnel.

A tetravalent meningococcal conjugate vaccine for serogroups A, C, W, and Y (MenACWY) is one of several recommended routine vaccinations for all new U.S. military recruits during enlisted basic training or officer accession training. Military recruits at all basic training centers routinely receive five or more concomitant vaccinations, including combination vaccines, within the first week of arrival at training facilities. While the safety and immunogenicity of coadministered vaccines in infants and children have been widely studied (1–9), the same approach has not been systematically followed for immunizations in adults and, in particular, for meningococcal polysaccharide conjugate vaccines in military personnel.

The effects on immunogenicity of concomitant vaccine administration are variable and may be vaccine specific. For example, inactivated polio vaccine (IPV) given alone or coadministered with other vaccines in military personnel showed that seroconversions to polio were more likely when multiple vaccines were administered (10). In contrast, it has been reported that immune response to the concomitant administration of the tetanus-diphtheria-acellular pertussis (Tdap) vaccine and the trivalent influenza vaccine was reported to be noninferior to sequential administration despite lower concentrations of antibody to tetanus, diphtheria, and pertussis toxin components (11, 12). West et al. reported that antibodies to rubella virus and pertussis filamentous hemagglutinin were below predicted levels in healthy adults who received concurrent administration of the bivalent *Haemophilus influenzae* type b (Hib)-hepatitis B vaccine (HBV) with priming doses of the diphtheria, tetanus, acellular pertussis (DTaP) vaccine and a booster dose of DTaP, oral polio vaccine, IPV, or measles, mumps, and rubella (MMR) vaccine (13). Bar-On et al. carried out a meta-analysis of 18 studies comparing the immune responses to and safety of concomitant and sequential administration of DTaP-Hib-HBV vaccines (14). They reported significantly lower immunological responses to Hib and HBV vaccines and more-localized reactions when these vaccines were coadministered.

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Similarly, the response to anti-polyribosyl ribitol phosphate (PRP) was lower in children when DTaP-IPV-Hib (diphtheria-tetanus-acellular pertussis/inactivated polio vaccine/*Haemophilus influenzae* type b vaccine) was given with a conjugate meningococcal vaccine (4). In addition, a combination of pneumococcal and meningococcal-conjugate vaccines administered to infants showed reduced meningococcal serogroup C immunogenicity compared with that obtained with a monovalent serogroup C meningococcal conjugate vaccine alone (7).

Immunogenicity data depending on the type of vaccines given concomitantly can be found in studies of a MenACWY vaccine (Menactra; Sanofi Pasteur, Swiftwater, PA, USA) in infants, in which no interference of MenACWY was seen with the antibody responses to the MMR vaccine but apparent interference was found with pneumococcal 7- to 13-valent conjugate vaccines (15). Burrage et al. (16) found carrier-induced epitopic suppression shown as decreased immunogenicity due to prior or concomitant administration of tetanus toxoid (TT)-containing vaccines, but not by the diphtheria toxin (CRM_{197}) carrier vaccines, on MenC-TT conjugates in children up to 18 years of age. On the other hand, in adolescents, serum bactericidal antibody (SBA) for 3 of the 4 meningococcal serogroups was higher when MenACWY (CRM_{197} carrier) was given concomitantly with Td than when given alone (15).

Based on the preponderance of the evidence and the potential for antigenic or carrier competition, we hypothesized that cohorts of individuals who had been concomitantly administered one or more vaccines along with MenACWY would have lower immune responses than a cohort of individuals receiving MenACWY alone. The study used an observational retrospective cohort design with preexisting military personnel data and specimens. The participants ranged in age from 17 to 43 years. Study endpoints were (i) specific antibody IgG concentrations, (ii) rabbit complement serum bactericidal antibody assay (rSBA) titers, and (iii) frequencies of responders reaching predetermined thresholds for immunological response (IgG $\geq$ 2 μg/ml; rSBA titer $\geq$ 8) and seroconversion for meningococcal serogroups C and Y.

MATERIALS AND METHODS

Sera. The Naval Health Research Center (NHRC) Institutional Review Board approved the study protocol (NHRC.2011.0015), and the work was carried out in accordance with the World Medical Association International Code of Medical Ethics for experiments involving humans. The approved study protocol was also reviewed by the Centers for Disease Control and Prevention (CDC) Human Research Protection Office, which determined it to be exempt from full review at CDC.

Serum samples were obtained from the Department of Defense Serum Repository (DoDSR), where they had been collected and stored at ≤ -30°C as part of a routine surveillance activity of active-duty U.S. military personnel. Baseline blood samples were collected during the initial screening of recruits. The mean time from baseline blood sample to vaccination was 4 months and ranged between 0 days and 19.4 months for all the participants (5 participants with >600 days from baseline to vaccination were excluded from analysis). All serum samples were blinded to the laboratory operators at CDC. Serum samples were labeled with a unique study number and shipped on dry ice to CDC for serological testing. Upon arrival, all samples were assigned a second unique CDC identifier number and stored at ≤ -70°C until testing. Once testing was complete, all serum samples were returned to NHRC for permanent storage.

Study design. There were four study groups, each including the MenACWY (Menactra; Sanofi Pasteur, Inc., Swiftwater, PA, USA) vaccine. Group 1 comprised 41 participants who had received only a single dose of MenACWY (designated MenACWY only). Group 2 consisted of 24 participants who had received MenACWY and Tdap (designated Plus Tdap only). Group 3 included 105 participants who had received MenACWY and four other vaccines among those (see Table 2) that were neither Tdap nor IPV (designated Plus 4 not Tdap/IPV). Group 4 comprised 150 participants who had received MenACWY and four other vaccines that did not include Tdap (designated Plus 4 not Tdap).

All participants had one serum sample drawn before their vaccination(s) and a second serum sample drawn during one of three postvaccination time windows: 1 to 2 months (51 participants), 3 to 5 months (187 participants), or 6 to 12 months (85 participants) as part of their routine health examinations. All participants had entered the military service between 2006 and 2012. Demographic data included age, sex, race/ethnicity, military branch, and military grade. No personal identifiable information was collected.

Assays. Serum antibody responses to *Neisseria meningitidis* serogroups C and Y were evaluated because of the higher incidence of these vaccine serogroups among adults living in the United States and in the military (17) than of other vaccine serogroups (18).

Measurement of antimeningococcal antibodies. Serum samples were analyzed for meningococcal serogroup-specific capsular polysaccharide-specific immunoglobulin G (IgG) antibodies using a multiplex Luminex technique (19) with some modifications. Each assay plate included a meningococcal human standard reference serum, CDC1992, diluted 2-fold for 12 dilutions starting at a titer of 1:25, and two internal quality control (QC) sera, 900268 and 900385, diluted 2-fold for 4 dilutions starting at a titer of 1:100. Test serum samples were diluted 2-fold for 6 dilutions starting at a titer of 1:25. IgG-free human serum (Sigma, St. Louis, MO, USA), and assay buffer blanks were included in each assay plate as reagent controls. Replicates were maintained for standard and QC serum samples and blanks. Four concordant serum dilutions with an interdilution coefficient of variance (CV) of $\leq$10% were used for acceptance criteria. Samples with overall CVs of >20% were retested. Data abstraction and analysis were performed using MasterPlex CT/QT software (MiraiBio Inc., San Francisco, CA, USA). The concentration of meningococcal serogroup-specific IgG in test sera was calculated relative to the human standard reference serum CDC1992 (20).

Measurement of serum bactericidal antibody titers. Serum bactericidal antibody titers to *N. meningitidis* serogroups C and Y were measured by rSBA using the target *N. meningitidis* strains C11 and S-1975, respectively (21, 22). Serum samples were serially diluted (3-fold) in Hanks' balanced salt solution (Gibco, Grand Island, NY, USA). The reaction time for bactericidal activity in the presence (25%, vol/vol) of a rabbit complement (Pel-Freez, Rogers, AR, USA) for strain C11 was 1 h at 37°C and ambient air, and for strain S-1975 it was 1 h at 37°C and 5% CO_2. Viability counts in tilt brain heart infusion agar plates (number 3710; CDC, Atlanta, GA, USA) were determined after 16 to 18 h of incubation by means of an automated colony counter (Synbiosis ProtoCOL 2; Synbiosis USA, Frederick, MD). Interpolated continuous titers were calculated as the serum dilution effecting 50% killing compared with the T_{60} growth control plate average as previously described (23).

Statistical analysis. Analyses were performed on IgG concentrations and rSBA titers of antimeningococcal antibodies to both serogroups C and Y. Thus, there were four distinct hypothesis tests (IgG-serogroup C, IgG-serogroup Y, rSBA-serogroup C, rSBA-serogroup Y) done for each of the three outcome measures: quantitative change in concentration/titer, change from below immunological thresholds at baseline to at or above the threshold postvaccination, and seroconversion.

The threshold for immunological response was defined as (i) an IgG concentration of $\geq$2 μg/ml (24, 25) or (ii) an rSBA titer of $\geq$8 and alternatively $\geq$128 (26, 27). Seroconversion was defined as a 2-fold increase in IgG concentration or a 4-fold increase in rSBA titer (28). A conservative rSBA titer of 4 was used for the calculation of seroconversion percentages.

Chi-square tests were used to examine differences in proportions of seroconversion between the MenACWY-only group and the other three

TABLE 1 Subject demographics and study characteristics[a]

Demographic group or characteristic	Mean no. (%) of subjects				
	All groups	MenACWY only, Grp 1	MenACWY Plus Tdap only, Grp 2	MenACWY Plus 4 not Tdap/IPV, Grp 3	MenACWY Plus 4 not Tdap, Grp 4
Total no. of subjects	320	41	24	105	150
Sex					
Female	80 (25)	9 (22)	7 (29)	13 (12)	51 (34)
Male	240 (75)	32 (78)	17 (71)	92 (88)	99 (66)
Race/ethnicity					
White	214 (67)	31 (76)	18 (75)	74 (70)	91 (61)
Black	56 (17)	4 (10)	1 (4)	14 (13)	37 (25)
Hispanic	27 (9)	2 (5)	0 (0)	12 (11)	13 (9)
Asian	10 (3)	1 (2)	2 (8)	2 (2)	5 (3)
NS	13 (4)	3 (7)	3 (13)	3 (3)	3 (2)
Age group (yr; range, 17 to 43)					
17–20	166 (52)	15 (37)	3 (13)	72 (69)	76 (51)
21–25	111 (35)	17 (41)	7 (29)	30 (29)	57 (38)
≥26	43 (13)	9 (25)	14 (58)	3 (3)	17 (11)
Mean (yr)	21.6	23.4	25.8	20.0	21.5
Months postvaccination					
Mean	4.96	4.6	3.54	5.44	4.95
Range	1–12	1–12	1–8	1–9	1–11

[a] NS, race/ethnicity was either "not specified," "other," or Native American; there were too few cases of each for analysis; Grp, group; IPV, inactivated polio vaccine; MenACWY, tetravalent meningococcal conjugate vaccine A, C, W, and Y; Tdap, tetanus-diphtheria-acellular pertussis vaccine.

groups combined. Logistic regressions with odds ratios (ORs) were used to test the associations of individual vaccine groups with seroconversion and with change among participants who were below the response threshold at baseline to whether they were at or above threshold postvaccination. Pearson's correlation coefficient (*r*) was used to test the relationship within and between the serological assays used in this study.

IgG concentrations and rSBA titers were base 2 log transformed prior to data analysis. Geometric mean concentrations (GMC) and geometric mean titers (GMT) were then calculated from the transformed values. Samples below the rSBA limit of detection were assigned a titer of 1.33 for GMT calculation. Kruskal-Wallis tests were used to analyze the change in IgG concentrations and rSBA titers. Statistical analyses were performed with SPSS (IBM SPSS Statistics for Windows, version 19.0.; Armonk, NY, USA).

The study was originally powered at 80% with a two-sided alpha of 0.05 to observe a 25% difference in effect sizes between the MenACWY-only group and each of the other three vaccine groups. However, the numbers of samples available for all the groups but the Plus 4 not Tdap group were smaller than expected. Nevertheless, *post hoc* we found effect sizes large enough that the number available was sufficient for the analysis.

As there were four hypothesis tests within each of the three outcome analyses, for each analysis we made a Bonferroni adjustment of the alpha levels to 0.0125. The within-test alpha value was set at 0.05.

RESULTS

The DoDSR provided baseline and postvaccination serum pairs for 325 U.S. military personnel with a mean age of 21.6 years (Table 1). Male participants constituted 75% (243/323) of the study population, most of whom were white (67%), followed by black (17%) and Hispanic (9%). The serum repository identified recipients of vaccine combinations consisting of 2 or 5 vaccines (specified by Health Level Seven code) from a pool of 15 possible vaccine combinations (Table 2). Eighty-five percent had been vaccinated with MenACWY between 2006 and 2008; the median month of entry was May 2007 for the MenACWY-only group and June 2007 for the participants who received MenACWY plus four other vaccines. Five participants had a prolonged time from baseline to postvaccination sera (>20 months) and were excluded from the final analysis, bringing the total number of participants to 320.

IgG concentrations and rSBA titers. Among the vaccine

TABLE 2 Vaccines that appeared at least 20 times in the combinations of 5 vaccines, sorted by CVX code[a]

Vaccine(s)	CVX code(s)	Occurrences (% of all combinations)
Measles, mumps, and rubella	003	99 (35)
Tetanus and diphtheria toxoids, adsorbed for adult use	009	190 (67)
Poliovirus vaccine, inactivated	010	153 (54)
Pneumococcal vaccine	033	61 (22)
Hepatitis A vaccine, adult dosage	052	127 (45)
Hepatitis A and hepatitis B vaccine	104	121 (43)
All vaccines, including hepatitis A vaccine	052, 104	248 (88)
Influenza virus vaccine-split virus (including purified surface antigen)	015	154 (54)
Influenza, unspecified	088	12 (4)
Novel influenza H1N1	127	12 (4)
All influenza vaccines	015, 088, 111, 127	252 (89)
Influenza virus vaccine, live, attenuated, intranasal use (FluMist)	111	74 (26)
Tetanus-diphtheria-pertussis	115	24 (9)
All vaccines containing tetanus	009, 115	216 (76)
Meningococcal conjugate ACWY	114	323 (100)

[a] CVX codes are international standards for transfer of clinical data between hospitals.

TABLE 3 Mean change in antimeningococcal IgG geometric mean concentrations and rSBA geometric mean titers from baseline to postvaccination per vaccine group[a]

Vaccine group	No. of subjects	IgG GMC (μg/ml)						rSBA GMT					
		Serogroup C			Serogroup Y			Serogroup C			Serogroup Y		
		Baseline	Post-vax	Mean change	Baseline	Post-vax	Mean change	Baseline	Post-vax	Mean change	Baseline	Post-vax	Mean change
Group 1 (ref group), MenACWY only	41	0.09	0.24	2.8	0.95	2.68	2.8	7.53	123.69	16.4	39.45	105.45	2.7
Group 2, Plus Tdap only	24	0.03	2.22	82.3	2.09	35.72	17.1	4.22	1284.65	304.2	21.46	632.16	29.5
Group 3, Plus 4, not Tdap/IPV	105	0.03	0.66	21.9	1.32	14.02	10.7	10.98	348.64	34.4	17.28	436.29	25.4
Group 4, Plus 4, not Tdap	150	0.04	1.28	33.0	1.21	14.97	12.4	4.12	392.43	95.8	23.43	1036.92	44.7
P value[b]				0.003			0.016[c]			0.001			0.002

[a] Abbreviations: GMC, geometric mean concentration (μg/ml); GMT, geometric mean titer; IgG, immunoglobulin G; IPV, inactivated polio vaccine; MenACWY, tetravalent meningococcal conjugate vaccine A, C, W, and Y; Post-vax, postvaccination; rSBA, rabbit complement serum bactericidal antibody assay; ref, reference; Tdap, tetanus-diphtheria-acellular pertussis vaccine.

[b] Kruskal-Wallis *P* values were calculated for testing the null hypothesis that the distribution of values across the four vaccine groups was the same.

[c] This *P* value is not statistically significant with a Bonferroni adjustment to the alpha level of 0.0125.

groups, group 1 (MenACWY only) had the lowest postvaccination IgG GMC (0.24 μg/ml and 2.68 μg/ml for serogroups C and Y, respectively) and the lowest rSBA GMT (124 and 106 for serogroups C and Y, respectively) of all the vaccination groups (Table 3). Mean changes in the IgG and rSBA values from baseline to postvaccination were also the lower for group 1 than for the other vaccine groups (Table 3). The highest mean change in IgG was observed in group 2 (Plus Tdap only), with 82.3 and 17.1 for serogroups C and Y, respectively. This group also had the highest mean change for serogroup C rSBA (304.2), while group 4 had the highest mean change (44.7) for serogroup Y. All *P* values were <0.004 except for the serogroup Y IgG ($P = 0.016$, not significant at the alpha level of 0.0125).

Change from below-response thresholds at baseline to the threshold level or above after vaccination. For this outcome, only participants whose baseline measures were below immunological response thresholds (threshold for IgG, ≥2 μg/ml; for rSBA titer, ≥8) were included. The percentages of participants who were below each immunological threshold at baseline but were at or above it postvaccination ("threshold status change") are shown in Table 4. Of all the vaccine groups, group 1 (MenACWY only) had the lowest percentages of participants with threshold status change for both assays and both serogroups (24% to 53%). Group 2 (Plus Tdap only) had the highest percentages of threshold status changes for serogroup C (68% for IgG and 95% for rSBA), and group 3 (Plus 4, not Tdap) had the highest percentages for serogroup Y (90% for IgG and 81% for rSBA).

These observations were echoed by the majority (7 of 12) of the odds ratios for groups 2, 3, and 4 that were statistically significant and above 1.0 (significant ORs ranged from 1.5 to 21.7) using group 1 as a reference (Table 4). Additionally, among the nonsignificant odds ratios, none of the point estimates were below 1, suggesting that groups 2, 3, and 4 in all cases had a greater chance of change in threshold status than did group 1. The congruency of these findings provides reassurance that a type II error did not occur.

The overall model *P* values were significant at the alpha level of 0.0125 for three of the four serogroup immunoassay models (Table 4), the exception being serogroup Y rSBA ($P = 0.114$). In addition, the majority (8 of 12) of independent group 2, 3, and 4 comparisons to group 1 were significant (*P* values < 0.05) (Table 4).

Response thresholds at baseline and postvaccination. Figure 1 shows the proportions of participants meeting the various immunological thresholds at baseline and postvaccination. Group 1 (MenACWY only) exhibited a lower response (below the threshold) than any other vaccine group for serogroup-specific IgG of ≥2 μg/ml (Fig. 1A) and rSBA titer (Fig. 1B shows the ≥8 threshold, and Fig. 1C shows the ≥128 threshold).

It should be noted that there were individuals who met the response threshold at baseline but had dipped below it postvaccination. Hence, it was possible for a group's overall percentage of meeting the response threshold to stay the same between baseline and postvaccination yet show a positive change in meeting the response threshold. For example, as shown in Fig. 1B, for serogroup Y rSBA in the MenACWY-only group, the baseline and postvaccination percentages were about the same, yet a threshold status change was observed (Table 4).

Pearson's *r* values between the IgG and rSBA immunoassays for serogroups C and Y at baseline were 0.33 and 0.24 and at postvaccination were 0.32 and 0.29, respectively (all *P* values were <0.001). Pearson's *r*'s between serogroups C and Y were 0.20 at baseline and 0.22 at postvaccination for IgG and were 0.23 and 0.22 for rSBA, respectively (all *P* values were <0.001).

Seroconversion. Seroconversion was calculated based on a 2-fold increase in meningococcal serogroup-specific IgG concentration or a 4-fold increase in rSBA titer as shown in Table 5. Group 1 (MenACWY only) had the lowest percentages of seroconversions for both serogroups (39% to 46%). The majority (9 of 12) of the odds ratios for groups 2, 3, and 4 were statistically significant and above 1.0 (significant ORs ranged from 1.8 to 4.8) using group 1 as the reference category (Table 5). Additionally, among the nonsignificant odds ratios, none of the point estimates were below 1, suggesting that groups 2, 3, and 4 in all cases had a greater chance of threshold status change than did group 1. The

TABLE 4 Numbers and percentages of subjects in each vaccine group who were below the IgG and rSBA immunologic thresholds at baseline and at or above the thresholds postvaccination[a]

Assay and vaccine group	Serogroup C						Serogroup Y					
	n^b	AT *n*	AT%	OR	95% CI	*P* value[c]	n^b	AT *n*	AT%	OR	95% CI	*P* value[c]
IgG												
Group 1 (ref group), MenACWY only	33	8	24	1.00 (ref)	**0.007**	23	10	43	1.00 (ref)	**<0.001**		
Group 2, Plus Tdap only	22	15	68	6.7	2.0–22	**0.002**	10	8	80	5.2	0.9–30	0.066
Group 3, Plus 4, not Tdap/IPV	90	29	32	1.5	0.6–3.7	0.394	50	36	72	3.3	1.2–9.4	**0.022**
Group 4, Plus 4, not Tdap	133	56	42	2.3	1.0–5.4	0.064	79	71	90	12	3.8–35	**<0.001**
rSBA												
Group 1 (ref group), MenACWY only	30	14	47	1.00 (ref)	**0.001**	17	9	53	1.00 (ref)	0.114		
Group 2, Plus Tdap only	20	19	95	22	2.6–183	**0.005**	14	10	71	2.2	0.5–1.0	0.297
Group 3, Plus 4, not Tdap/IPV	65	50	77	3.8	1.5–9.6	**0.004**	60	47	78	3.2	1.0–10	**0.043**
Group 4, Plus 4, not Tdap	116	92	79	4.4	1.9–10	**0.001**	79	64	81	3.8	1.3–11	**0.018**

[a] Unadjusted logistic regressions with odds ratios are shown for the association of the MenACWY-only group and each of the other vaccine groups. Significant *P* values are in bold; the alpha was 0.05 for the significance of the parameter values within each model. Abbreviations: AT *n* and AT%, number and percentage, respectively, of subjects who were below the immunologic threshold at baseline and at or above the threshold postvaccination; CI, confidence interval; IgG, immunoglobulin G; IPV, inactivated polio vaccine; MenACWY, tetravalent meningococcal conjugate vaccine A, C, W, and Y; OR, odds ratio; rSBA, rabbit complement serum bactericidal assay; ref, reference; Tdap, tetanus-diphtheria-acellular pertussis vaccine.

[b] *n* includes only subjects with baseline concentrations/titers below immunologic thresholds. The immunologic threshold was defined as a change in IgG concentration of ≥2 μg/ml or rSBA titer of ≥8.

[c] Overall *P* value for the variable "vaccine group." This tested the null hypothesis that vaccine group was a significant predictor of change in attaining threshold. The alpha was adjusted to 0.0125 to account for the four hypothesis tests.

congruency of these findings provides reassurance that a type II error did not occur.

The overall model *P* values were significant at the alpha level of 0.0125 for three of the four serogroup immunoassay models (Table 5), the exception being serogroup Y rSBA ($P = 0.042$). In addition, the majority (9 of 12) of the independent group 2, 3, and 4 comparisons to group 1 were significant (*P* values < 0.05) (Table 5).

The lower percentages of seroconversions in group 1 relative to the other groups can also be seen in Fig. 2. *P* values from chi-

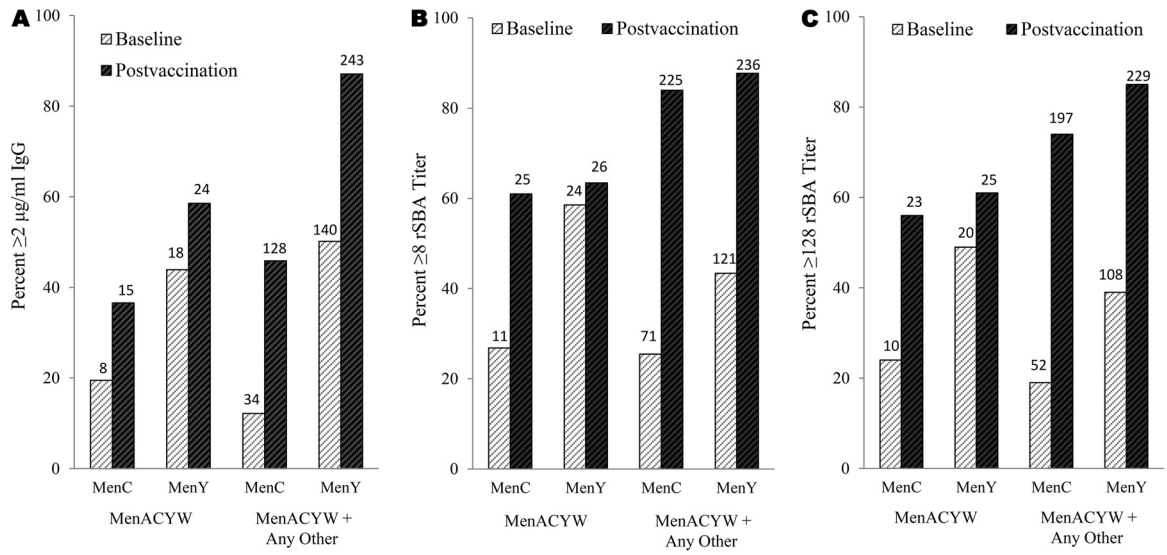

FIG 1 Each panel compares the MenACWY-only group to the other three vaccine groups combined and the percentages of participants who reached the immunologic threshold at baseline and at postvaccination for the immunoglobulin G (IgG) assay, with a ≥2-μg/ml IgG threshold (A), the rabbit-complement serum bactericidal assay (rSBA) titer threshold of ≥8 (B), and the rSBA titer threshold of ≥128 (C). MenC, serogroup C; MenY, serogroup Y.

TABLE 5 Numbers and percentages of subjects in each vaccine group who seroconverted[a]

Assay and vaccine group	No. of subjects in group	Serogroup C					Serogroup Y				
		SC *n*	SC%	OR	95% CI	*P* value[b]	SC	SC%	OR	95% CI	*P* value[b]
IgG											
Group 1 (ref group), MenACWY only	41	16	39	1.0 (ref)	**0.002**	16	39	1.0 (ref)	**<0.001**		
Group 2, Plus Tdap only	24	18	75	4.7	1.5–14	**0.007**	17	71	3.8	1.3–11	**0.016**
Group 3, Plus 4 not Tdap/IPV	105	67	64	2.8	1.3–5.8	**0.008**	72	69	3.4	1.6–7.2	**0.001**
Group 4, Plus 4 not Tdap	150	113	75	4.6	2.2–9.5	**<0.001**	113	75	4.8	2.3–9.9	**<0.001**
rSBA											
Group 1 (ref group), MenACWY only	41	19	46	1.0 (ref)	0.016[c]	17	41	1.0 (ref)	0.042[c]		
Group 2, Plus Tdap only	24	19	79	4.4	1.4–14	**0.012**	15	63	2.3	0.8–6.6	0.105
Group 3, Plus 4 not Tdap/IPV	98	61	62	1.9	0.9–4.0	0.086	55	56	1.8	0.9–3.8	0.117
Group 4, Plus 4 not Tdap	146	103	71	2.7	1.4–5.6	**0.005**	96	66	2.7	1.3–5.5	**0.006**

[a] Unadjusted logistic regressions with odds ratios are shown for the association of the MenACWY-only group and each of the other vaccine groups. Seroconversion was defined as a 2-fold increase in IgG concentration (μg/ml) or a 4-fold increase in rSBA titer (titers below the lower limit of detection were assigned a conservative titer of 4). Significant *P* values are in bold; the alpha was 0.05 for the significance of the parameter values within each model. Abbreviations: CI, confidence interval; IgG, immunoglobulin G; IPV, inactivated polio vaccine; MenACWY, tetravalent meningococcal conjugate vaccine A, C, W, and Y; OR, odds ratio; rSBA, rabbit complement serum bactericidal assay; ref, reference; SC *n* and SC%, number and percentage, respectively, of subjects who seroconverted; Tdap, tetanus-diphtheria-acellular pertussis vaccine.

[b] Overall *P* values for the logistic regression. This tested the null hypothesis that vaccine group was a significant predictor of seroconversion.

[c] This *P* value is not statistically significant with a Bonferroni adjustment to the alpha level of 0.0125. The alpha was adjusted to 0.0125 to account for the four hypothesis tests.

square tests of group 1 versus the combination of groups 2, 3, and 4 were significant at the alpha level of 0.0125 except for the IgG assay for serogroup Y ($P = 0.0126$).

The correlations between the IgG and SBA immunoassays were low for serogroups C and Y and ranged from 0.20 to 0.23 (all *P* values were <0.001).

DISCUSSION

Concomitant or near-concomitant administration of vaccines offers many benefits: ease of administration, fewer number of shots if comixed, fewer clinic visits, and hence better compliance. Several studies have investigated the immunogenicity and safety of coadministered vaccines with conflicting outcomes. Considering the variations in responses to vaccines in relation to the sequence of administration and the necessity to establish a robust immune response, this study was undertaken to evaluate the immune response to the MenACWY vaccine when administered concomitantly with other vaccines in military recruits.

The administration of the MenACWY vaccine elicited antibodies to *N. meningitidis* serogroups C and Y, evidenced by significant increases in serum IgG concentrations, functional bactericidal antibodies, and percentages of individuals who seroconverted following vaccination. In this study, IgG GMCs, rSBA GMTs, and percentages of participants with threshold status change and with seroconversion were significantly higher in the study groups receiving MenACWY concomitantly with other vaccines than in the control group receiving MenACWY alone. Contrary to our initial expectation of immunological interference against the MenACWY vaccine when administered with additional vaccines, we found that total (IgG) and functional (rSBA) immune responses had significantly greater increases, both categorically (attaining the response threshold and seroconversion) and absolutely (concentration and continuous titer), than with MenACWY alone.

The findings here are in agreement with those found in a study of IPV administered alone or along with multiple other vaccines in military personnel (10), despite the fact that the concomitant vaccines varied from those evaluated in this study. In the IPV study, inclusion of Tdap resulted in a significant increase in the number of seroconversions in the study group receiving multiple vaccinations (>4 vaccines). However, in the present study, the frequency of seroconversion was greater for any combination of vaccines with MenACWY, regardless of the inclusion of Tdap. Additional systems biology studies similar to those conducted for meningococcal vaccines and other adult vaccines may help explain the potential immune-stimulatory effects triggered during simultaneous immunization with a variety of antigens (29). Because we were measuring only the response to MenACWY, and because the number of participants for each vaccine in combination was not consistent, we could not determine the effects of individual vaccines.

Eighty-four percent of the participants entered between 2006 and 2009, 1 to 3 years after the recommendation from the Advisory Committee on Immunization Practices in May 2005 for routine MenACWY immunizations in adolescents (30). It is possible that some of these military personnel had already been vaccinated with a meningococcal vaccine prior to recruitment, thereby contributing to higher baseline antibody levels for serogroups

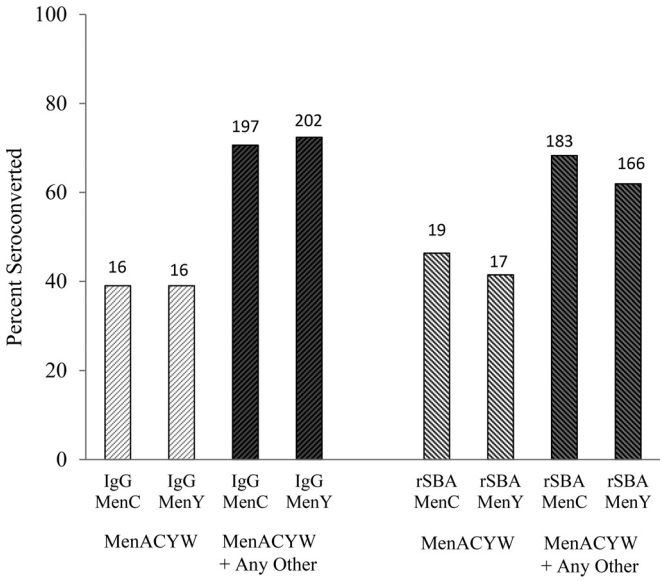

FIG 2 The percentage of seroconversions in the MenACWY-only group is compared to that of the other three vaccine groups combined. The group of 4 columns on the left show the immunoglobulin G (IgG) assay, in which seroconversion was defined as a 2-fold rise in concentration from baseline to postvaccination. The group of 4 columns on the right shows the rabbit complement serum bactericidal antibody assay (rSBA), in which seroconversion was defined as a 4-fold rise in titer from baseline to postvaccination. A titer of 4 was assigned to values below the lower limit of detection. MenC, serogroup C; MenY, serogroup Y.

C and Y. We have previously reported that the immune response to both conjugated and unconjugated meningococcal serogroup C polysaccharide in U.S. military personnel wanes significantly from 6 months to 3 years following vaccination (23). It is possible that prior administration of the MenACWY vaccine may have had a nonquantifiable effect on the postvaccine measures due to anamnestic response.

This study contributes to the evaluation of concomitant vaccination practices in adults, specifically among military personnel. Given the study design, we cannot attribute directly the effects of vaccinations on the immune response and cannot, for example, make a direct comparison to a study showing a suppression of the immune response to meningococcal vaccine as previously observed in a pediatric population (31).

Whether the associations observed are a function of enhanced immunologic responses in individuals due to the kind and number of vaccinations received concomitantly awaits further investigation. In previous studies, enhancements of the immune response could be attributed to adjuvanticity from concomitant vaccines (32) or immune stimulation by vaccine antigens of multiple biochemical compositions (29). Systems biology studies in adults have indicated that distinct immunological pathways are stimulated by different vaccines (including meningococcal, yellow fever, and influenza vaccines), resulting in distinct molecular signatures and mechanisms for antibody response (29). So, it is possible that concomitant vaccines stimulate multiple immune mechanism(s), leading to an increase in the immune response to a target vaccine. Additional prospective randomized clinical trials controlling for confounders in this population will be useful to confirm these observations.

This study has some limitations. First, some vaccine groups were smaller than anticipated, potentially limiting the number of comparisons yielding significantly predictive differences between the groups. Second, this is a retrospective study, making it subject to selection bias. The authors were unable to control for all causal factors such as prior MenACWY immunization before the participants arrived at the camp. However, we found only 13 participants (evenly distributed across all groups) with high levels of antibodies at baseline for both serogroups. MenACWY was recommended by the ACIP in May 2005 (33), when most of our participants were past the recommended vaccination age, and initial coverage in the population was low (34). Third, the period between baseline serum collection and vaccination at camp varied widely (mean, 4.96 months). Group 2 (MenACWY Plus Tdap only) had a mean baseline-to-vaccination time of 3.54 months, possibly increasing the chance for higher antibody measurements in this group. Patel et al. have shown a decline in levels of antibody to MenACWY depending on the time postvaccination, although that study did not control for MenACWY administration along with other vaccines (23).

Despite these limitations, this study found a positive effect of concomitant vaccination on the immune response to MenACWY in this unique target population. Additional studies should be undertaken to examine the immunological outcome of other bacterial and viral vaccines when administered along with multiple vaccinations. Such studies, combined with statistical modeling, may provide an opportunity to select the best combination of vaccines to ensure a synergistic immunological outcome and better seroprotection in adults.

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We declare that we have no conflicts of interest.

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REFERENCES

1 **CDC Advisory Committee for Immunization Practices.** 22 March 2016, posting date. Vaccine recommendations of the ACIP. http://www.cdc.gov/vaccines/hcp/acip-recs. Accessed 3 April 2014.

2. **Godfroid F, Denoel P, de Grave D, Schuerman L, Poolman J.** 2004. Diphtheria-tetanus-pertussis (DTP) combination vaccines and evalua-

tion of pertussis immune responses. Int J Med Microbiol **294:**269–276. http://dx.doi.org/10.1016/j.ijmm.2004.07.007.
3. **Halperin SA, Pianosi K.** 2010. Immunization in Canada: a 6-year update. J Can Chiropr Assoc **54:**85–91.
4. **Kitchin NR, Southern J, Morris R, Hemme F, Thomas S, Watson MW, Cartwright K, Miller E.** 2007. Evaluation of a diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b vaccine given concurrently with meningococcal group C conjugate vaccine at 2, 3 and 4 months of age. Arch Dis Child **92:**11–16. http://dx.doi.org/10.1136/adc.2005.076109.
5. **Mills E, Gold R, Thipphawong J, Barreto L, Guasparini R, Meekison W, Cunning L, Russell M, Harrison D, Boyd M, Xie F.** 1998. Safety and immunogenicity of a combined five-component pertussis-diphtheria-tetanus-inactivated poliomyelitis-Haemophilus B conjugate vaccine administered to infants at two, four and six months of age. Vaccine **16:**576–585. http://dx.doi.org/10.1016/S0264-410X(97)00241-7.inelevel00.
6. **Rennels M, American Academy of Pediatrics Committee on Infectious Diseases.** 2005. Prevention and control of meningococcal disease: recommendations for use of meningococcal vaccines in pediatric patients. Pediatrics **0116:**0496–505. http://dx.doi.org/10.1542/peds.2005-1314.
7. **Buttery JP, Riddell A, McVernon J, Chantler T, Lane L, Bowen-Morris J, Diggle L, Morris R, Harnden A, Lockhart S, Pollard AI, Cartwright K, Moxon ER.** 2005. Immunogenicity and safety of a combination pneumococcal-meningococcal in infants—a randomized controlled trial. JAMA **293:**1751–1758. http://dx.doi.org/10.1001/jama.293.14.1751.
8. **Kitchin N, Southern J, Morris R, Hemme F, Cartwright K, Watson M, Miller E.** 2006. A randomised controlled study of the reactogenicity of an acellular pertussis-containing pentavalent infant vaccine compared to a quadrivalent whole-cell pertussis-containing vaccine and oral poliomyelitis vaccine, when given concurrently with meningococcal group C conjugate vaccine to healthy UK infants at 2, 3 and 4 months of age. Vaccine **24:**3964–3970. http://dx.doi.org/10.1016/j.vaccine.2006.02.018.
9. **Kitchin NR.** 2011. Review of diphtheria, tetanus and pertussis vaccines in clinical development. Expert Rev Vaccines **10:**605–615. http://dx.doi.org/10.1586/erv.11.60.
10. **Broderick MP, Oberste MS, Moore D, Romero-Steiner S, Hansen CJ, Faix DJ.** 2015. Effect of multiple, simultaneous vaccines on polio seroresponse and associated health outcomes. Vaccine **33:**2842–2848. http://dx.doi.org/10.1016/j.vaccine.2014.07.088.
11. **McNeil SA, Noya F, Dionne M, Predy G, Meekison W, Ojah C, Ferro S, Mills EL, Langley JM, Halperin SA.** 2007. Comparison of the safety and immunogenicity of concomitant and sequential administration of an adult formulation tetanus and diphtheria toxoids adsorbed combined with acellular pertussis (Tdap) vaccine and trivalent inactivated influenza vaccine in adults. Vaccine **25:**3464–3474. http://dx.doi.org/10.1016/j.vaccine.2006.12.047.
12. **Weston WM, Chandrashekar V, Friedland LR, Howe B.** 2009. Safety and immunogenicity of a tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine when co-administered with influenza vaccine in adults. Hum Vaccin **5:**858–866. http://dx.doi.org/10.4161/hv.9961.
13. **West DJ, Rabalais GP, Watson B, Keyserling HL, Matthews H, Hesley TM.** 2001. Antibody responses of healthy infants to concurrent administration of a bivalent haemophilus influenzae type b-hepatitis B vaccine with diphtheria-tetanus-pertussis, polio and measles-mumps-rubella vaccines. BioDrugs **15:**413–418. http://dx.doi.org/10.2165/00063030-200115060-00007.
14. **Bar-On ES, Goldberg E, Fraser A, Vidal L, Hellmann S, Leibovici L.** 2009. Combined DTP-HBV-HIB vaccine versus separately administered DTP-HBV and HIB vaccines for primary prevention of diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae B (HIB). Cochrane Database Syst Rev **2009**(3)**:**CD005530. http://dx.doi.org/10.1002/14651858.CD005530.pub2.
15. **Sanofi Pasteur.** 2014. 284 Menactra. Final label—26 August 2014. http://www.fda.gov/downloads/BiologicBloodVaccines/Vaccines/ApprovedProducts/UCM131170.pdf. Accessed 20 January 2016.
16. **Burrage M, Robinson A, Borrow R, Andrews N, Southern J, Findlow J, Martin S, Thornton C, Goldblatt D, Corbel M, Sesardic D, Cartwight K, Richmond P, Miller E.** 2002. Effect of vaccination with carrier protein on response to meningococcal C conjugate vaccines and value of different immunoassays as predictors of protection. Infect Immun **70:**4946–4954. http://dx.doi.org/10.1128/IAI.70.9.4946-4954.2002.
17. **Broderick MP, Faix DJ, Hansen CJ, Blair PJ.** 2012. Trends in meningococcal disease in the United States military, 1971-2010. Emerg Infect Dis **18:**1430–1437. http://dx.doi.org/10.3201/eid1809.120257.
18. **Centers for Disease Control.** Accessed 9 December 2013. Active Bacterial Core Surveillance (ABCs) report. Emerging Infections Program Network. Neisseria meningitidis, 2012. http://www.cdc.gov/abcs/reports-findings/survreports/mening12.pdf.
19. **Lal G, Balmer P, Stanford E, Martin S, Warrington R, Borrow R.** 2005. Development and validation of a nonaplex assay for the simultaneous quantitation of antibodies to nine Streptococcus pneumoniae serotypes. J Immunol Methods **296:**135–147. http://dx.doi.org/10.1016/j.jim.2004.11.006.
20. **Elie CM, Holder PK, Romero-Steiner S, Carlone GM.** 2002. Assignment of additional anticapsular antibody concentrations to the Neisseria meningitidis group A, C, Y, and W-135 meningococcal standard reference serum CDC1992. Clin Diagn Lab Immunol **9:**725–726. http://dx.doi.org/10.1128/CDLI.9.3.725-726.2002.
21. **Borrow R, Carlone GM.** 2001. Serogroup B and C serum bactericidal assays. Methods Mol Med **66:**289–304. http://dx.doi.org/10.1385/1-59259-148-5:289.
22. **Maslanka SE, Gheesling LL, Libutti DE, Donaldson KB, Harakeh HS, Dykes JK, Arhin FF, Devi SJ, Frasch CE, Huang JC, Kriz-Kuzemenska P, Lemmon RD, Lorange M, Peeters CC, Quataert S, Tai JY, Carlone GM.** 1997. Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. The Multilaboratory Study Group. Clin Diagn Lab Immunol **4:**156–167.
23. **Patel M, Romero-Steiner S, Broderick MP, Thomas CG, Plikaytis BD, Schmidt DS, Johnson SE, Milton AS, Carlone GM, Clark TA, Messonnier NE, Cohn AC, Faix DJ.** 2014. Persistence of serogroup C antibody responses following quadrivalent meningococcal conjugate vaccination in United States military personnel. Vaccine **32:**3805–3809. http://dx.doi.org/10.1016/j.vaccine.2014.05.001.
24. **Gold R, Lepow ML, Goldschneider I, Draper TF, Gotshlich EC.** 1979. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. J Infect Dis **140:**690–697. http://dx.doi.org/10.1093/infdis/140.5.690.
25. **Maslanka SE, Tappero JW, Plikaytis BD, Brumberg RS, Dykes JK, Gheesling LL, Donaldson KB, Schuchat A, Pullman J, Jones M, Bushmaker J, Carlone GM.** 1998. Age-dependent Neisseria meningitidis serogroup C class-specific antibody concentrations and bactericidal titers in sera from young children from Montana immunized with a licensed polysaccharide vaccine. Infect Immun **66:**2453–2459.
26. **Gheesling LL, Carlone GM, Pais LB, Holder PF, Maslanka SE, Plikaytis BD, Achtman M, Densen P, Frasch CE, Kayhty H.** 1994. Multicenter comparison of Neisseria meningitidis serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. J Clin Microbiol **32:**1475–1482.
27. **Andrews N, Borrow R, Miller E.** 2003. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. Clin Diagn Lab Immunol **10:**780–786.
28. **Keyserling H, Papa T, Koranyi K, Ryall R, Bassily E, Bybel MJ, Sullivan K, Gilmet G, Reinhardt A.** 2005. Safety, immunogenicity, and immune memory of a novel meningococcal (groups a, c, y, and w-135) polysaccharide diphtheria toxoid conjugate vaccine (mcv-4) in healthy adolescents. Arch Pediatr Adolesc Med **159:**907–913. http://dx.doi.org/10.1001/archpedi.159.10.907.
29. **Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, Schmidt DS, Johnson SE, Milton A, Rajam G, Kasturi S, Carlone GM, Quinn C, Chaussabel D, Palucka AK, Mulligan MJ, Ahmed R, Stephens DS, Nakaya HI, Pulendran B.** 2014. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat Immunol **15:**195–204. http://dx.doi.org/10.1038/ni.2789.
30. **Centers for Disease Control and Prevention.** 2010. Estimated vaccination coverage, with ≥1 dose of MCV4 vaccine among adolescents aged 13-17 years by race/ethnicity and by state and local area. National Immunization Survey—Teen, United States, 2008. CDC, Atlanta, GA. http://www.cdc.gov/vaccines/imz-managers/coverage/nis/teen/data/tables-2008.html#overall. Accessed 22 December 2014.
31. **Borrow R, Dagan R, Zepp F, Hallander H, Poolman J.** 2011. Glycoconjugate vaccines and immune interactions, and implications for vaccination schedules. Exp Rev Vaccines **10:**1621–1631. http://dx.doi.org/10.1586/erv.11.142.

32. **Aimanianda V, Haensler J, Lacroix-Desmazes S, Kaveri SV, Bayry J.** 2009. Novel cellular and molecular mechanisms of induction of immune responses by aluminum adjuvants. Trends Pharm Sci **30:**287–295. http://dx.doi.org/10.1016/j.tips.2009.03.005.
33. **CDC Advisory Committee for Immunization Practices.** 2005. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep **54**(RR–7)**:**1–21.
34. **CDC Advisory Committee for Immunization Practices.** 2010. Summary report, October 27-28, 2010, Atlanta, Georgia. CDC, Atlanta, GA. http://www.cdc.gov/vaccines/acip/meetings/downloads/min-archive/min-oct10.pdf.