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Immunologic Hyporesponsiveness to Serogroup C but Not Serogroup A following Repeated Meningococcal A/C Polysaccharide Vaccination in Saudi Arabia

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In Saudi Arabia, vaccination with the meningococcal A/C polysaccharide (MACP) vaccine is advised every 3 years. A clinical outcome study was performed to test the effect of repeat vaccination with the MACP vaccine on the immune responses among Saudi nationals who live in the Makkah and Jeddah areas. Subjects ($n = 230$) aged 10 to 29 years were selected: 113 subjects with two or more prior vaccinations with the MACP vaccine, 79 subjects with one prior vaccination with the MACP vaccine, and 38 subjects naïve to vaccination with the MACP vaccine. All subjects received the MACP vaccine in 2002, and serum bactericidal antibody (SBA) titers were measured before and 1 month after vaccination with the MACP vaccine. For serogroup C, geometric mean SBA titers 1 month following vaccination with the MACP vaccine were 708.6 (95% confidence interval [CI], 217.5 to 2,308.9) for those naïve to prior vaccination with the MACP vaccine, and they were significantly higher ($P < 0.0001$) than 25.0 (95% CI, 12.4 to 50.2) for those who had received one prior vaccination with the MACP vaccine and 32.4 (95% CI, 18.7 to 56.4) for those who had received two or more doses of the MACP vaccine. For serogroup A, the geometric mean SBA titer 1 month after receipt of the MACP vaccine was 1,649.3 (95% CI, 835.2 to 3,256.9) for those naïve to prior vaccination, and the titers were lower ($P = 0.67$) than 2,185.7 (95% CI, 1,489.4 to 3,207.7) for those who had received one prior dose of the MACP vaccine and significantly lower ($P = 0.042$) than 3,540.8 (95% CI, 2,705.2 to 4,634.5) for those who had received two or more doses of the MACP vaccine. For serogroup C, the proportions of nonresponders (SBA titers, <8) were 19% for the naïve cohort, 52% for the cohort with one prior vaccination, and 49% for the cohort with two or more prior vaccinations. Following repeated doses of the MACP vaccine, hyporesponsiveness to serogroup C is evident, with high percentages of MACP vaccinees having SBA titers below the putative protective SBA titer. Serogroup A responses following vaccination with the MACP vaccine were boosted. Introduction of the serogroup C conjugate vaccine would provide long-term protection against serogroup C disease; however, quadrivalent conjugate vaccines are required to provide long-time protection against disease caused by serogroups A, W135, and Y.

The annual pilgrimage during the Hajj and Umrah seasons results in more than 4 million visitors to Saudi Arabia. Such mass population movements have been associated with outbreaks of a number of infectious diseases, including meningococcal diseases (1, 2, 23, 31, 32). For more than two decades the Ministry of Health of Saudi Arabia has advised both residents and pilgrims to receive the meningococcal A/C polysaccharide (MACP) vaccine, an approach which reflects the effectiveness of the meningococcal polysaccharide vaccine in controlling disease outbreaks (4, 5).

Following a Hajj-related outbreak of disease caused by serogroup W135 in 2001, the Saudi Arabian meningococcal control program was expanded in 2002 to include the quadrivalent meningococcal serogroup A, C, W135, and Y vaccine for those coming for Hajj or Umrah (6) (present vaccination requirements are continually published in the *Weekly Epidemiological Record*).

MACP vaccines have proved effective for the short-term control of disease caused by both serogroups A (13, 21, 22, 33) and C (7, 14, 16). The bivalent and quadrivalent meningococcal polysaccharide vaccines are T-cell independent and do not induce memory cells, necessitating repeat vaccinations at regular intervals.

Concerns about the induction of immunologic hyporesponsiveness after repeat vaccination with the polysaccharide vaccine have been confirmed in previous studies of the meningococcal serogroup C polysaccharide vaccine (18, 19, 27, 29, 37), whereas the results of studies of the serogroup A vaccine have conflicted (10, 15, 17, 26, 29, 38).

The Saudi Arabian requirement for meningococcal polysaccharide vaccination among local residents offered the oppor-

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tunity to confirm the occurrence of immunologic hyporesponsiveness after repeat serogroup C polysaccharide vaccination and to explore the effects of repeated serogroup A polysaccharide vaccination.

MATERIALS AND METHODS

Study design and subject selection. The present study was a clinical outcome study, with subjects recruited on a prospective basis. All subjects were given the meningococcal polysaccharide vaccine as part of the routine Saudi Arabian meningococcal control program.

Study population. The study population was randomly chosen from attendees of vaccination clinics in the Makkah and Jeddah areas based either in a general practice or schools, depending on the age group and the location of the subject. In Saudi Arabia the school class correlated to age, as follows: years 1 to 6, ages 6 to 11 years, respectively; years 7 to 9, ages 12 to 14 years, respectively; and years 10 to 12, ages 15 to 17 years, respectively.

Subjects were included if they were Saudi nationals aged 10 to 29 years (inclusive) and capable of giving written consent for participation. Subjects were excluded if they had been vaccinated against meningococcal disease outside Saudi Arabia or just prior to enrollment. Those who had received another vaccine(s) within the previous 4 weeks and those who were scheduled to receive another vaccine(s) 4 weeks post-meningococcal vaccination were excluded. Pregnant females were also excluded, as were subjects with acute febrile illness or immunosuppression from treatment or underlying disease. Subjects who were unable to attend for the second visit were also excluded.

Finally, subjects were classified into three groups according to their history of previous meningococcal polysaccharide vaccination. Data were analyzed according to the previous immunization status of the study subjects. Two research visits were scheduled. Visit 1 included prevaccination blood sampling and vaccination. Visit 2 included postvaccination blood sampling.

Vaccines. The two vaccines used (Mencevax AC and Mencevax ACWY) were purchased from GlaxoSmithKline, Jeddah, Saudi Arabia. Both vaccines are lyophilized preparations of purified polysaccharide from *Neisseria meningitidis* of serogroups A and C for the first vaccine and of serogroups A, C, W135, and Y for the second vaccine. Each 0.5-ml dose of reconstituted vaccine contains 50 μg of serogroup A polysaccharide and 50 μg of serogroup C polysaccharide dissolved in an isotonic sodium chloride solution. The diluent supplied contains 0.25% phenol as a preservative.

Serology. All blood samples were centrifuged at 3,000 rpm (Centaur 2 MSE instrument; Sanyo) for 10 min. The serum was isolated, and the samples were then labeled and frozen at −80°C. The samples were shipped to the Manchester Public Health Laboratory Service, Manchester, United Kingdom, for serogroup-specific enzyme-linked immunosorbent assay (ELISA) and serum bactericidal antibody (SBA) titer determination.

SBA titers against strain F8238 (serogroup A) and strain C11 (serogroup C) were determined as described previously (30) by using baby rabbit serum (Pel-Freeze Incorporated, Rodgerson, Ariz.) as an exogenous complement source. SBA titers were expressed as the reciprocal of the final serum dilution giving ≥50% killing after 60 min for serogroup C and 90 min for serogroup A. For computational purposes titers <4 were assigned a value of 2. A reevaluation of correlates of protection for serogroup C previously showed that protection correlates with an SBA titer of between 8 and 64 (8); however, recently, by use of United Kingdom efficacy data, an SBA titer ≥8 has now been shown to be a more appropriate cutoff (3).

Serogroup A- and C-specific immunoglobulin G (IgG) ELISAs were performed as described previously (12, 20). The lower limit of the assay was 0.1 μg/ml; serum samples with antibody levels <0.1 μg/ml were assigned a value of 0.05 μg/ml.

Statistical analysis. Antibody levels were log transformed for analysis. geometric mean (GM) SBA titers and GM IgG concentrations with 95% confidence intervals (CIs) were calculated. The SPSS package (version 10.1) was used for statistical analysis of the data for both serogroup A and serogroup C.

A paired *t* test was used to test for changes in responses between time points within groups. Differences between groups were compared by one-way analysis of variance and post hoc tests.

The percentage of nonresponders to the vaccine was calculated by using an SBA titer of <8. The McNemar nonparametric test was carried out to compare the proportion of subjects who presented with SBA titers ≥8 prior to vaccination to the proportion of subjects who presented with SBA titers ≥8, ≥32, and ≥ 128 1 month following vaccination.

TABLE 1. Population allocation and recruitment

Site	No. of subjects				
	Total	Who agreed to participate	Who were eligible	Who presented to first visit	Who presented to second visit
School 1	525	107	82	70	61
School 2	493	65	46	42	37
School 3	336	59	31	31	30
Medical school	300	55	42	42	39
General practitioner	147	100	45	45	36
Total	1,801	386	246	230	203

RESULTS

Population characteristics. Table 1 summarizes the total numbers of subjects allocated, eligible, and participating in the study.

A history of previous vaccination was considered the dependent variable; accordingly, the subjects were classified into three major groups to compare the outcomes: 38 subjects were naïve to prior vaccination, 79 subjects had previously received one vaccination, and 113 subjects had previously received two or more vaccinations.

Of the 192 subjects who had previously been vaccinated, 136 subjects had been vaccinated within the previous 3 years and 56 subjects had been vaccinated ≥3 years earlier.

The subjects were classified into four age groups. The numbers of subjects by age group and gender are shown in Table 2.

Serogroup A. For serogroup A, significant increases in the GM SBA titers were demonstrated from before to 1 month after receipt of the initial dose of the meningococcal polysaccharide vaccine among the three groups ($P < 0.0001$). Among the naïve subjects there was a 26-fold increase in the GM SBA titer 1 month following vaccination. The other groups had three- to fourfold increases in GM SBA titers 1 month after vaccination; however, the prevaccination titer was high in comparison with the serogroup C prevaccination titer for the same groups (Table 3).

The 1 month postvaccination GM SBA titer was significantly higher for subjects who had previously been vaccinated two or more times than for subjects who were naïve to prior vaccination with the MACP vaccine ($P = 0.042$) but not significantly higher than the GM SBA titer for subjects who had been vaccinated once before ($P = 0.127$). Subjects who had been vaccinated once before had a 1.3-fold higher titer than subjects who were naïve to prior vaccination ($P = 0.678$) (Table 3).

TABLE 2. Study population classified by age and gender

Age range	No. of subjects		
	Female	Male	Total
10–14	0	35	35
15–19	14	98	112
20–24	29	12	41
25–29	26	16	42
Total	69	161	230

As was the case for the GM SBA titer, at 1 month following vaccination there were significant increases in the IgG concentrations (as determined by ELISA) among the three study groups ($P < 0.0001$). However, at 1 month postvaccination naïve subjects showed significantly lower IgG concentrations (as determined by ELISA) than subjects who had previously been vaccinated either once or two or more times ($P = 0.03$) (Table 3).

An SBA titer ≥8 was used to identify responders. Among the subjects who were naïve to prior vaccination there was a significant increase in the percentage of responders ($P = 0.016$) from prevaccination to 1 month after vaccination. On the other hand, among the subjects who had been vaccinated either once or two or more times there was a very high percentage of subjects with titers ≥8 prior to vaccination, and hence, the percentage of responders had not significantly increased 1 month following vaccination ($P = 0.219$ and 0.125, respectively).

Data were also analyzed by age, gender, smoking status, and period since the last vaccination. None of the four factors had a significant effect on the differences in the responses to vaccination (data not shown).

Serogroup C. For serogroup C, significant increases in GM SBA titers were demonstrated among the three groups from before to 1 month following meningococcal polysaccharide vaccination (naïve subjects, $P < 0.0001$; subjects vaccinated once, $P = 0.018$; subjects vaccinated two or more times, $P < 0.0001$). The naïve subjects had an increased GM SBA titer of more than 35-fold 1 month postvaccination, whereas GM SBA titers increased only about 2- to 3-fold postvaccination for the other two groups. Subjects naïve to vaccination showed significantly higher GM SBA titers 1 month following vaccination compared to those for subjects previously vaccinated one or more times ($P < 0.0001$) (Table 4).

As for the GM SBA titer, there were significant increases in IgG concentrations (as determined by ELISA) 1 month following vaccination among the three groups in the study ($P < 0.0001$). Subjects naïve to prior vaccination with the MACP vaccine had a 10-fold increase in the IgG concentration 1 month following vaccination. The GM IgG concentrations 1 month postvaccination were not significantly different among the three groups ($P = 0.567$) (Table 4).

Responders were identified as those subjects with SBA titers of 8. A significant increase in the percentage of responders was identified among the subjects naïve to vaccination ($P = 0.002$). The increase in the percentages of responders among the subjects vaccinated once before was not significant ($P = 0.143$), whereas among subjects previously vaccinated two or more times, the increases in the percentages of responders by use of cutoff levels of ≥8 and ≥32 were significant ($P = 0.008$ and 0.031, respectively) but not by use of a cutoff level of ≥128 ($P = 0.523$) (Table 4).

Data were also analyzed by age, gender, smoking status, and period since last vaccination. None of the four factors had a significant effect on differences in the responses to vaccination (data not shown).

DISCUSSION

This study has demonstrated a difference in the immune responses to serogroups A and C after repeated vaccination

TABLE 3. GM serogroup A SBA titers, percentage of responders, and GM serogroup A-specific IgG concentrations

History of previous vaccination[a]	GM SBA titer (95% CI)		GM IgG concn (μg/ml [95% CI])[b]		No. (%) of subjects with:			
	Prior to vaccination	1 mo postvaccination	Prior to vaccination	1 mo postvaccination	SBA titer ≥8 prior to vaccination	SBA titer ≥8 1 mo after vaccination	SBA titer ≥32 1 mo after vaccination	SBA titer ≥128 1 mo after vaccination
Naïve to vaccination	62.6 (24.5–160.4)	1,649.3 (835.2–3,256.9)	7.1 (4.3–11.9)	41.5 (26.6–64.5)	22 (69)	31 (97)	31 (97)	30 (94)
Previously vaccinated once	575.7 (320.8–967.4)	2,185.7 (1,489.4–3,207.7)	32.8 (23.0–46.4)	91.2 (62.5–133.8)	60 (92)	63 (98)	63 (98)	62 (97)
Previously vaccinated two or more times	1,219.8 (835.4–1,781.1)	3,540.8 (2,705.2–4,634.5)	43.8 (33.6–56.9)	86.9 (66.5–113.7)	99 (96)	100 (100)	99 (99)	99 (99)

[a] The numbers of subjects in the pre- and postvaccination groups were 32 and 32, respectively, for the naïve cohort; 65 and 64, respectively, for the cohort vaccinated once; and 103 and 100, respectively, for the cohort vaccinated two or more times.
[b] The concentrations were determined by ELISA.

TABLE 4. Serogroup C GM SBA titers, percentage of responders, and serogroup C-specific GM IgG concentrations

History of previous vaccination[a]	GM SBA titer (95% CI)		GM IgG concn (μg/ml [95% CI])[b]		No. (%) of subjects with:			
	Prior to vaccination	1 mo postvaccination	Prior to vaccination	1 mo postvaccination	SBA titer ≥8 prior to vaccination	SBA titer ≥8 1 mo after vaccination	SBA titer ≥32 1 mo after vaccination	SBA titer ≥128 1 mo after vaccination
Naïve to vaccination	19.0 (7.4–49.1)	708.6 (217.5–2,308.9)	3.8 (1.8–7.7)	38.8 (26.6–64.5)	13 (41)	26 (81)	25 (78)	23 (72)
Previously vaccinated once	11.3 (6.4–19.7)	25.0 (12.4–50.2)	13.8 (10.1–19.0)	27.2 (18.8–39.4)	24 (37)	31 (48)	28 (44)	23 (36)
Previously vaccinated two or more times	14.0 (8.4–23.2)	32.4 (18.7–56.4)	17.7 (13.1–24.1)	30.8 (22.7–41.8)	40 (39)	52 (51)	50 (50)	44 (44)

[a] The numbers of subjects in the pre- and postvaccination groups were 32 and 32, respectively, for the naïve cohort; 65 and 64, respectively, for the cohort vaccinated once; and 103 and 101, respectively, for the cohort vaccinated two or more times.
[b] The concentrations were determined by ELISA.

with the meningococcal polysaccharide vaccine in Saudi residents aged 10 to 29 years.

This is the first published report of a study measuring the functional antibody response to demonstrate an increase in the titer of SBA to the serogroup A polysaccharide following repeated polysaccharide vaccination in individuals in this age group. Postvaccination, the serogroup A GM SBA titer in subjects who had previously received two or more doses of polysaccharide vaccine was higher than the titer in those receiving it for the first time ($P = 0.042$). The number of responders (SBA titer, ≥8) increased from 69% in the naïve group to 100% in the postvaccination group after at least two doses. Serogroup A-specific GM IgG concentrations also increased following vaccination. The highest IgG concentration 1 month after vaccination was detected among those who had been vaccinated two or more times.

The results of this study support those of other studies (15, 17, 26) that have indicated that repeated doses of serogroup A meningococcal polysaccharide vaccine induce sustained levels of antibody to the serogroup A polysaccharide. However, the three previous studies (15, 17, 26) showed an induction of the immune response to serogroup A only by ELISA. Conversely, two studies (10, 29) demonstrated immunologic hyporesponsiveness to repeated vaccination with the serogroup A meningococcal polysaccharide vaccine. Those two studies measured both IgG concentrations (by ELISA) and SBA titers (10, 29).

Despite the use of bactericidal antibody assays together with the IgG concentration measurements, the conflicting results between this study and the two previous studies (10, 29) are obvious. The variation in results could be related to a number of different factors. First, the population sample size used in this study is much larger than those used in the previous two studies. Second, the populations used in the previous two studies were from different countries: Gambia (29) and the United Kingdom (10). Also, the Gambian study (29) recruited children aged 5 years during a vaccination campaign after an outbreak of serogroup A meningococcal disease. Those children had previously received two doses of the vaccine at the ages of 3 to 6 months and 18 to 24 months, respectively. The study conducted in the United Kingdom (10) recruited university students aged 18 to 25 years who had been vaccinated only once 6 months earlier as part of preventive measures taken after an outbreak. It is clear that the populations selected in this study were different in several aspects, such as the ages compared to the ages of the subjects evaluated in the Gambian study (children) and the time since the last vaccination compared to that in the study conducted in the United Kingdom. Third, the vaccine given in the United Kingdom and Gambia (Pasteur Merieux) was produced by a company different from that which produced the vaccine used in the present study (GlaxoSmithKline). Finally, and most importantly, in this study the subjects were local residents of Makkah and Jeddah, where the rate of natural exposure could have been similar to that for Gambian children but completely different from that for the subjects evaluated in the United Kingdom, where serogroup A is very rarely reported. Furthermore, the subjects in the present study were potentially exposed to serogroup A and W135 meningococci during the Hajj 2000 outbreak, when both serogroups were isolated from case patients (28).

Avidity indices have previously been used to investigate the

maturation of long-lived B cells following vaccination with meningococcal serogroup A and C polysaccharide and conjugate vaccines (9, 24, 25, 36). Antibody avidity, the strength with which a multivalent antibody binds to a complex antigen, increases over time following primary immunization with a T-cell-dependent antigen but decreases following immunization with T-cell-independent antigens. Joseph et al. (25) demonstrated that following serogroup A polysaccharide immunization the avidity indices remained significantly above the prevaccination levels 1 year following vaccination among children 2 to 3 years of age, whereas after serogroup C polysaccharide vaccination, the avidity indices declined significantly postvaccination among children (9). These findings also indicate that the serogroup A polysaccharide may not be behaving as a classical T-cell-independent antigen.

The proportions of putative responders postvaccination with the serogroup C polysaccharide were 81% for the naïve group and 48% for those who had received one prior polysaccharide vaccine. As shown in previously published studies, repeated doses of polysaccharide vaccine resulted in immune hyporesponsiveness (19, 27, 29, 37).

Repeated vaccination with the serogroup A and C polysaccharide vaccine gives reduced functional antibody responses to the serogroup C portion of the vaccine but increased responses to the serogroup A portion of the vaccine among residents of the cities of Makkah and Jeddah in Saudi Arabia aged 10 to 29 years. The reasons for this difference in response are unclear, but other bacterial polysaccharides have been reported to act in a T-cell-dependent fashion (34).

Use of the conjugate vaccine should be considered for long-term protection against serogroup C disease. However, other serogroups (A, Y, and W135) will continue to cause problems, and trials with the quadrivalent meningococcal conjugate vaccine have commenced (11, 35). Once it is licensed, use of the quadrivalent meningococcal conjugate vaccine in Saudi Arabia will provide long-term protection and will be more cost-effective.

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Written informed consent was obtained for all participants in this study. Ethical approval was obtained from the Saudi Arabian Ministry of Health; the Umm Al-Qura University of Makkah, Makkah, Saudi Arabia; the Public Health Laboratory Service, Colindale, London, United Kingdom; and Manchester University, Manchester, United Kingdom.

The trial was conducted according to International Committee for Harmonization Guidelines for Good Clinical Practice and with appropriate quality assurance.

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