

Meningococcal Group C and W135 Immunological Hyporesponsiveness in African Toddlers[∇]

Helen Findlow,^{1*} Samba Sow,² Ray Borrow,^{1,3} Milagritos Tapia,² Fadima Cheick Haidara,²
Adebayo K. Akinsola,⁴ Olubukola T. Idoko,⁴ Fatoumata Diallo,² Richard Adegbola,⁴
Yuxiao Tang,⁵ Varsha Parulekar,⁶ Helen Chadha,¹ Lesley Mabey,¹ Daniel Holme,¹
Kelly Townsend,¹ Julie Chaumont,⁵ F. Marc LaForce,⁵ Prasad S. Kulkarni,⁷
Elisa Marchetti,⁵ Simonetta Viviani,⁵ Musa Hassan-King,⁵
and Marie-Pierre Preziosi⁸

Vaccine Evaluation Unit, Health Protection Agency North West, Manchester Royal Infirmary, Manchester, United Kingdom¹;
Centre pour le Développement des Vaccins (CVD Mali), Bamako, Mali²; University of Manchester, Inflammation Sciences
Research Group, School of Translational Medicine, Stopford Building, Manchester M13 9PL, United Kingdom³;
Medical Research Council Laboratories, Basse, The Gambia⁴; Meningitis Vaccine Project (MVP), PATH,
Ferney-Voltaire, France⁵; DiagnoSearch Life Sciences Pvt. Ltd., Mumbai, India⁶; Serum Institute of
India Ltd., Pune, India⁷; and MVP, Initiative for Vaccine Research,
World Health Organization (WHO), Geneva, Switzerland⁸

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A phase II clinical study was conducted in African toddlers (aged 12 to 23 months), with subjects receiving either investigational meningococcal group A conjugate (PsA-TT), meningococcal ACWY polysaccharide (PsACWY), or *Haemophilus influenzae* type b (Hib-TT) vaccine. Ten months following vaccination, the 3 study groups were further randomized to receive a dose of PsA-TT, a 1/5 dose of PsACWY, or a dose of Hib-TT vaccine. Group A serum bactericidal antibody (SBA) results have been reported previously, with PsA-TT demonstrating superior immunogenicity versus PsACWY vaccine. Immunogenicity for serogroups W135 and C was assessed by SBA assay to investigate the impact of multiple doses in this age group. Blood samples were taken prior to vaccination, 28 days and 40 weeks post-primary vaccination, and 7 and 28 days post-booster vaccination with a 1/5 dose of PsACWY. Subjects who had previously received a full dose of PsACWY had W135 SBA geometric mean titers (GMTs) of 26.1 and 4.4 at 7 and 28 days post-booster vaccination with a 1/5 PsACWY dose, respectively, whereas the W135 SBA GMTs of naïve subjects at these time points following vaccination with a 1/5 dose of PsACWY were 861.1 and 14.6, respectively. Similar differences were observed for serogroup C, with SBA GMTs of 99 and 5.9 at 7 and 28 days post-booster vaccination with a 1/5 dose of PsACWY, respectively, for naïve subjects, compared to 4.1 and 3.2 for previously vaccinated subjects. Immunologic hyporesponsiveness for groups C and W135 was observed following a full dose of PsACWY vaccine at 12 to 23 months of age and a 1/5 dose of PsACWY 10 months later compared to the case for PsACWY-naïve subjects receiving a 1/5 dose of PsACWY vaccine.

Outbreaks of *Neisseria meningitidis* group A recur frequently in the African meningitis belt, where they are responsible for high mortality and morbidity. Polysaccharide vaccines against group A have been available since the 1970s, but due to the limitations of these vaccines, polysaccharide-protein conjugate vaccines have been developed. In 2001, the Meningitis Vaccine Project (MVP), a partnership between the World Health Organization (WHO) and the Program for Appropriate Technology in Health (PATH), was created through core funding from the Bill & Melinda Gates Foundation, with the goal of eliminating group A meningococcal epidemics in sub-Saharan Africa through the development and use of a monovalent group A meningococcal conjugate vaccine. A phase I clinical study of a group A meningococcal conjugate vaccine, MenAfriVac

(PsA-TT; Serum Institute of India Ltd. [SIIL]), was carried out successfully with adult volunteers in India (7). Phase II and II/III clinical studies were performed in 1- to 29-year-olds in Africa and India, and a single dose of PsA-TT was found to be well tolerated, safe, and highly immunogenic compared to a group A polysaccharide vaccine (15).

A phase II clinical study was conducted in African toddlers (aged 12 to 23 months) to assess the safety and immunogenicity of a single dose of PsA-TT. Immunogenicity was assessed against that of a licensed meningococcal polysaccharide ACWY (PsACWY) vaccine, and the safety profile was compared to those for PsACWY and *Haemophilus influenzae* type b (Hib-TT) conjugate vaccines. Immunologic memory and persistence of antibodies induced by a single dose of PsA-TT were assessed. Ten months following primary vaccination, the three study groups were further randomized into nine subgroups to receive either a full dose of PsA-TT or Hib-TT vaccine or a 1/5 dose of PsACWY vaccine. The PsA-TT vaccine was shown to demonstrate superior immunogenicity versus the group A component of the PsACWY vaccine for this age group. Antibody persistence was demonstrated over the 10 months follow-

* Corresponding author. Mailing address: Vaccine Evaluation Unit, Health Protection Agency North West, P.O. Box 209, Clinical Sciences Building, Manchester Royal Infirmary, Manchester M13 9WZ, United Kingdom. Phone: 44 161 276 6791. Fax: 44 161 276 6792. E-mail: helen.findlow@hpa.org.uk.

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ing PsA-TT vaccination, as was the induction of immunologic memory (15). Serum samples from these toddlers were also assayed to evaluate the serogroup C and W135 immune responses following one or two doses of PsACWY vaccine in this age group.

Vaccination with repeated doses of polysaccharide can induce the phenomenon of hyporesponsiveness, which is characterized by a decreased immune response upon repeated doses compared to the response after a single dose. Most of the current knowledge about hyporesponsiveness induced by meningococcal polysaccharide vaccines comes from experiences with group C vaccines. Induction of group C hyporesponsiveness has been observed in different age groups, including infants (4), toddlers (8), and adults (5, 6, 13, 14). The immune response to group W135 is thought to be similar to the response to group C. There are few data available on the immune response to multiple doses of group W135 polysaccharide. A study conducted in Uganda in those aged 2 to 19 years showed that revaccination with a full dose of PsACWY 1 year following an initial full dose resulted in hyporesponsiveness to groups W135 and Y (10). Reduced responses to the serogroup W135 portion of a meningococcal group ACWY conjugate vaccine were also shown in adolescents who had previously received the PsACWY vaccine (3, 6). Epidemics of group W135 meningitis have been reported from countries within the African meningitis belt where the only means of control is via a polysaccharide vaccine; therefore, data regarding the effects of repeated doses of vaccines containing W135 polysaccharide are required.

We report here on the immune responses of toddlers to groups C and W135 after a full dose of PsACWY at 12 to 23 months of age followed by a 1/5 dose 10 months later.

MATERIALS AND METHODS

Study group. Full details of the study group have been reported elsewhere (15); in brief, healthy toddlers (12- to 23-month-olds) who were fully vaccinated according to the local Expanded Program on Immunization (EPI) schedule were recruited from two urban quarters in Bamako, Mali, and a rural area in Basse, in the Upper River Region of The Gambia. The clinical trial is registered at www.controlled-trials.com under number SRCTN78147026.

Vaccines and vaccination. The PsA-TT vaccine is available as a lyophilized 10-dose vial to be reconstituted with a 5-ml diluent ampoule. A single 0.5-ml dose of the reconstituted PsA-TT vaccine contained 10 µg of purified *N. meningitidis* group A polysaccharide conjugated to 10 to 33 µg of tetanus toxoid (TT) carrier protein, with aluminum phosphate as an adjuvant, Tris (hydroxymethyl)aminoethane as buffer, 0.9% sodium chloride, 0.01% thimerosal preservative, and sterile water for injection (MenAfriVac investigational vaccine; SIIIL, Pune, India). A single 0.5-ml dose of PsACWY vaccine contained 50 µg of each meningococcal ACWY polysaccharide (Mencevax ACWY; GlaxoSmithKline [GSK], Belgium). A single dose of the reconstituted Hib-TT vaccine contained 10 µg of purified Hib polyribosylribitol phosphate (PRP) conjugated to 20 to 40 µg of tetanus toxoid (Hiberix; GSK). All initial doses of vaccine were administered intramuscularly in the right thigh. Booster doses of PsA-TT and Hib vaccine were administered intramuscularly in the right deltoid, and booster doses of a 1/5 dose of PsACWY were administered subcutaneously in the right deltoid.

Subjects were randomized at a 1:1:1 ratio to one of three groups to receive either PsA-TT, a licensed PsACWY reference vaccine, or control Hib-TT vaccine. Ten months following primary vaccination, subjects in each primary vaccination group were further randomized at a 1:1:1 ratio to receive a booster dose of PsA-TT or Hib-TT or one-fifth of a full dose of PsACWY and were monitored for the next 18 months.

The original study design had nine vaccine groups post-booster vaccination (15), but for the group C and W135 analysis, the nine groups were redistributed to create four groups (Table 1) depending upon whether the subjects had re-

TABLE 1. Vaccine groups analyzed in this study (redistributed depending on the number of doses of PsACWY vaccine received)^a

Group	No. of doses of PsACWY	Primary vaccination (12–23 mo of age)	Vaccination at 20–33 mo of age
1	None None None None	PsA-TT PsA-TT Hib-TT Hib-TT	PsA-TT Hib-TT PsA-TT Hib-TT
2	1 dose at 22–33 mo of age 1 dose at 22–33 mo of age	PsA-TT Hib-TT	1/5 dose of PsACWY 1/5 dose of PsACWY
3	1 dose at 12–23 mo of age 1 dose at 12–23 mo of age	PsACWY PsACWY	PsA-TT Hib-TT
4	2 doses, at 12–23 mo of age and 22–33 mo of age	PsACWY	1/5 dose of PsACWY

^a PsA-TT, group A conjugate vaccine; PsACWY, group A, C, W135, and Y polysaccharide vaccine; Hib-TT, *Haemophilus influenzae* type b conjugate vaccine.

ceived no doses of PsACWY vaccine, one dose of PsACWY vaccine at primary or booster vaccination, or two doses of PsACWY vaccine.

Data for all time points throughout the manuscript are presented for these four groups rather than the original three-group (following primary vaccination) and nine-group (following booster vaccination) study design presented for the *N. meningitidis* group A data (15).

Immunogenicity. Blood samples collected prior to the primary injection, 28 days and 40 weeks after primary vaccination, and 7 and 28 days following booster vaccination were assayed by a serum bactericidal antibody (SBA) assay for serogroups C and W135. At each time point, SBA assays were performed against a group C target strain, C11 (phenotype C:16:P1.7-1,1), and a group W135 strain, M01 240070 (phenotype W:NT:P1.18-1,3), as previously described by Maslanka et al. (9). The complement source used in the SBA assay was pooled sera from 3- to 4-week-old rabbits (Pel Freez Biologicals, WI). Titers were expressed as reciprocal serum dilutions yielding ≥50% killing after 60 min. The lower limit of detection was a titer of 4. Titers of <4 were assigned a value of 2 for geometric mean titer (GMT) analysis.

Data analysis. The SBA titers from each time point were log transformed, and geometric means with 95% confidence intervals (95% CI) were calculated. In addition to the calculation of GMTs, the proportion of subjects with SBA titers of ≥8, with 95% CI, were calculated. For group C, an SBA titer of ≥8 was defined as putatively protective (2).

Statistical significance between GMTs was calculated using a paired *t* test for differences between time points for a group, a two-sample *t* test for differences between two groups at a time point, and analysis of variance (ANOVA) for differences between the four groups at each time point.

RESULTS

Serogroup C and W135 SBA GMTs and 95% CI prevaccination, 28 days and 40 weeks after primary injection, and 7 and 28 days post-booster vaccination are shown in Fig. 1. The percentage of subjects with SBA titers of ≥8 (with 95% CI) for each group is shown in Table 2. The GMT for group 1, which did not receive any PsACWY vaccine, did not change over the study period.

Primary vaccination. Significant increases in the SBA GMTs for serogroups C and W135 from prevaccination to 28 days postvaccination (*P* < 0.001) were demonstrated for the two groups which received PsACWY vaccine (groups 3 and 4) at primary vaccination. At 28 days post-primary vaccination, there was a significant difference between the GMTs of the four groups (*P* = 0.010) for serogroup C, but there was no significant difference between the four groups for serogroup W135 (*P* = 0.658) (Fig. 1).

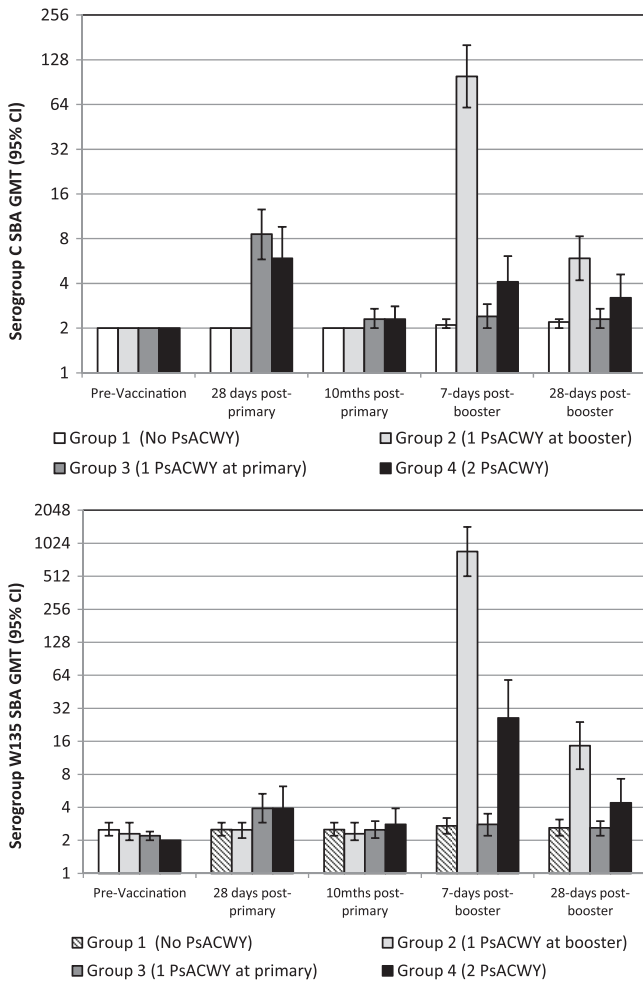


FIG. 1. Serogroup C and W135 SBA GMTs for the four groups prior to vaccination, 28 days and 10 months post-primary vaccination, and 7 and 28 days post-booster vaccination.

The percentages of subjects achieving SBA titers of ≥ 8 were low for both serogroups C and W135 (Table 2). Only 31.5% (95% CI, 23.7% to 40.3%) and 23.8% (95% CI, 14.0% to 36.2%) of subjects in groups 3 and 4, respectively, achieved SBA titers of ≥ 8 . For group W135, the percentages were 13.2% (95% CI, 7.9% to 20.3%) and 12.9% (95% CI, 5.7% to 23.9%) for groups 3 and 4, respectively.

Booster vaccination. Ten months following primary vaccination, the serogroup C and W135 GMTs for those who received PsACWY at primary vaccination (groups 3 and 4) declined back to baseline levels (Fig. 1). Two of the four groups, groups 2 and 4, received a 1/5 dose of PsACWY vaccine 10 months after the initial vaccination visit.

(i) Serogroup W135. Significant increases in the serogroup W135 SBA GMTs were demonstrated from before vaccination to 7 days and 28 days after a 1/5 dose of PsACWY for both groups 2 and 4 ($P < 0.02$). The SBA GMTs of the naïve subjects (group 2) were significantly higher than those of primed subjects (group 4) at day 7 ($P < 0.001$) and day 28 ($P < 0.001$) post-booster vaccination.

Among the subjects who were naïve to PsACWY (group 2),

TABLE 2. Percentages of subjects with SBA titers of ≥ 8

Time point	Serogroup C				Serogroup W135			
	Group 1 (no PsACWY)	Group 2 (1 PsACWY dose at booster vaccination)	Group 3 (1 PsACWY dose at primary vaccination)	Group 4 (2 PsACWY doses)	Group 1 (no PsACWY)	Group 2 (1 PsACWY dose at booster vaccination)	Group 3 (1 PsACWY dose at primary vaccination)	Group 4 (2 PsACWY doses)
Prevaccination	0 (0-1.4) (267)	0 (0-2.7) (134)	0 (0-2.7) (135)	0 (0-5.5) (65)	4.6 (2.4-7.9) (262)	2.3 (0.5-6.5) (133)	2.3 (0.5-6.5) (132)	0 (0-5.7) (63)
28 days post-primary vaccination	0 (0-1.4) (259)	0 (0-2.7) (134)	31.5 (23.7-40.3) (130)	23.8 (14-36.2) (63)	4.3 (2.2-7.6) (256)	4.5 (1.7-9.6) (132)	13.2 (7.9-20.3) (129)	12.9 (5.7-23.9) (62)
Ten months post-primary vaccination	0 (0-1.4) (256)	0 (0-2.7) (133)	3.0 (0.8-7.6) (132)	4.7 (1-13.1) (64)	4.7 (2.4-8) (256)	2.3 (0.5-6.5) (133)	4.5 (1.7-9.6) (132)	7.8 (2.6-17.3) (64)
7 days post-booster vaccination	1.2 (0.3-3.6) (241)	74.2 (65.6-81.6) (124)	4 (1.3-9.2) (124)	18.0 (9.4-30) (61)	5.8 (3.2-9.6) (240)	83.9 (76.2-89.9) (124)	5.6 (2.3-11.3) (124)	42.6 (30-55.9) (61)
28 days post-booster vaccination	2.1 (0.7-4.8) (240)	25.2 (17.9-33.7) (127)	3.2 (0.9-8.0) (125)	11.3 (4.7-21.9) (62)	4.6 (2.3-8.1) (240)	34.6 (26.4-43.6) (127)	7.2 (3.3-13.2) (125)	16.1 (8-27.7) (62)

there was a 374-fold rise in the SBA GMT from pre- to 7 days post-booster vaccination, which declined 59-fold by 28 days post-booster vaccination but was 6.3-fold higher than the level pre-booster vaccination. For the subjects who had received prior PsACWY vaccine (group 4), the fold differences were lower, with a 9.3-fold increase from pre- to 7 days post-booster vaccination which declined 5.9-fold by 28 days post-booster vaccination and was only 1.57-fold higher than the level pre-booster vaccination.

Among the subjects who were naïve to prior PsACWY vaccination (group 2), there was an increase in the percentage of subjects with SBA titers of ≥ 8 , from a pre-booster vaccination proportion of 2.3% (95% CI, 0.5% to 6.5%) to 83.9% (95% CI, 76.2% to 89.9%) at 7 days post-booster vaccination, which declined to 34.6% (95% CI, 26.4% to 43.6%) by 28 days post-booster vaccination (Table 2). Compared to subjects who had previously been vaccinated with PsACWY (group 4), the proportion of subjects with SBA titers of ≥ 8 increased from 7.8% (95% CI, 2.6% to 17.3%) prior to the second PsACWY vaccination to 42.6% (95% CI, 30% to 55.9%) at 7 days post-booster vaccination, with a decline to 16.1% (95% CI, 8% to 27.7%) by 28 days post-booster vaccination.

(ii) Serogroup C. The responses seen for serogroup C for the two groups (groups 2 and 4) which received PsACWY 40 weeks after the primary vaccination visit were similar to those observed for serogroup W135.

As for group W135, significant increases in the group C SBA GMTs were demonstrated from before vaccination to 7 days and 28 days after a 1/5 dose of PsACWY for both groups 2 and 4 ($P < 0.05$). The SBA GMTs of the naïve subjects (group 2) were significantly higher than those of primed subjects (group 4) at day 7 ($P < 0.001$) and day 28 ($P = 0.030$) post-booster vaccination.

Among the subjects who were naïve to PsACWY (group 2), there was a 49.5-fold rise in the SBA GMT from pre- to 7 days post-booster vaccination which declined 16.8-fold by 28 days post-booster vaccination but was 2.95-fold higher than that pre-booster vaccination. For the subjects who had received prior PsACWY vaccination (group 4), the fold differences were lower, with a 1.8-fold increase from pre- to 7 days post-booster vaccination which declined 1.3-fold by 28 days postvaccination and was only 1.4-fold higher than the level pre-booster vaccination.

Among the subjects who were naïve to prior PsACWY (group 2), there was an increase in the percentage of subjects with SBA titers of ≥ 8 , from the pre-booster vaccination proportion of 0% (95% CI, 0% to 2.7%) to 74.2% (95% CI, 65.6% to 81.6%) at 7 days post-booster vaccination, which declined to 25.2% (95% CI, 17.9% to 33.7%) by 28 days post-booster vaccination.

In the previously PsACWY-vaccinated group (group 4), the proportion of subjects with SBA titers of ≥ 8 increased from 4.7% (95% CI, 1% to 13.1%) prebooster to 18% (95% CI, 9.4% to 30%) at 7 days post-booster vaccination, with a decline to 11.3% (95% CI, 4.7% to 21.9%) by 28 days post-booster vaccination.

DISCUSSION

To our knowledge, this is the first study to demonstrate immunologic hyporesponsiveness to serogroup W135 and confirms previous reports of serogroup C immunologic hyporesponsiveness following a 1/5 dose of PsACWY vaccine in toddlers who had previously been vaccinated with a full dose of

PsACWY (10 months earlier) compared to naïve subjects who received only a 1/5 dose of PsACWY.

It is well documented that repeated doses of meningococcal C polysaccharide lead to immune hyporesponsiveness, but to date, no studies have reported hyporesponsiveness to W135 polysaccharide in this age group. It is well known that the immune systems of young children cannot process polysaccharide antigens in a manner for stimulating an effective response (16). Poor SBA responses 28 days following a full dose of PsACWY vaccine at 12 to 23 months of age were observed in this study. One month following a full dose of PsACWY vaccine, there was no significant difference between group W135 SBA GMTs of the four vaccine groups, highlighting the poor immunogenicity of the group W135 portion of the vaccine in this age group. The poor immune response observed in this age group is consistent with the response to two doses of the same vaccine in Saudi Arabian children aged < 18 months (1). In contrast to the results reported here and in the study conducted by Al-Mazrou et al. (1), an earlier Finnish study reported a large proportion of responders (a responder was defined as an individual showing a ≥ 4 -fold increase in SBA titer) following one dose of PsACWY vaccine in children of 6 to 23 months of age (11). For groups C and W135, 90% and 85% of subjects, respectively, were classified as responders.

Immunized persons with hyporesponsiveness can respond with serum antibody to revaccination with meningococcal polysaccharide or conjugate vaccine, although the magnitudes of their responses are lower than those for individuals of similar ages immunized for the first time. The clinical significance of hyporesponsiveness or the mechanism underlying this reduced antibody response is unknown but has been suggested to be due to B cells becoming anergic from continuous exposure to low doses of polysaccharide or to repeated vaccination stimulating naturally primed memory B cells to become antibody-producing plasma cells without regeneration of the memory B cell population. The group W135 SBA GMTs on days 7 and 28 following vaccination with a 1/5 dose of PsACWY vaccine in subjects who had received prior PsACWY vaccination (group 4) were significantly lower than the responses observed for PsACWY-naïve subjects (group 2).

Group W135 immunologic hyporesponsiveness has previously been reported for those over the age of 2 years at initial vaccination. A reduced response to a second full dose of PsACWY vaccine was observed in a Ugandan cohort (aged 2 to 19 years) compared to the response following the first dose, 1 year earlier (10). The group C response was also lower in the subjects who had received prior PsACWY vaccination than in naïve subjects, consistent with previously reported studies (8).

For both serogroups W135 and C, the SBA GMT of naïve individuals was significantly higher at 7 days postvaccination than that of subjects who had received prior PsACWY vaccination, and a significant decline was observed from day 7 to day 28. This decline was also true for the group W135 SBA GMT of those subjects who had received prior PsACWY vaccine, but the magnitude of the difference was much lower than that for naïve subjects. The group A responses of this cohort following PsA-TT or PsACWY vaccination were also higher on day 7 than on day 28 (15). The decline in SBA GMTs from day 7 to 28 may be attributed to the presence of group-specific IgM, but the serogroup A-specific IgG of this cohort was also

seen to decline significantly (15). Measurement of serogroup C- and W135-specific IgM and IgG in this cohort would provide further information on this observation. Similar findings were reported from two studies of a licensed meningococcal group A, C, W135, and Y conjugate vaccine in children aged 4 to 6 years (12) and in adolescents (6). Keyserling et al. (6) speculated that the higher SBA GMT observed at day 8 in adolescents could be due to an early onset of high-avidity IgG antibodies. Pichichero et al. (12) measured group-specific IgG and evaluated the IgG avidity via avidity indices. There were no differences in either IgG or the avidity indices measured on days 8 and 28 following vaccination for all groups.

The serogroup C and W135 SBA responses following polysaccharide vaccination have been shown to be age dependent. An age-dependent response to group W135 polysaccharide was evident in this study, with the SBA GMT 1 month following a 1/5 dose of PsACWY vaccine at 20 to 33 months of age being 3.7-fold higher than the response to a full dose at 12 to 23 months of age. The group W135 SBA GMTs at 10 months post-primary vaccination show that this difference in response is not attributable to natural immunity. The response to the group C component following a 1/5 dose was comparable to that after a full dose at a younger age. A previous study demonstrated that the immune responses to group C and W135 polysaccharides were poor up until the age of 4, with an age-dependent increase in the number of subjects with SBA titers of ≥ 8 (1).

This study confirms previously reported data showing that the immune responses to group A, C, and W135 polysaccharides differ (1, 5), as the group A response following a full dose of PsACWY at 12 to 23 months of age was considerably higher (15) than those seen here for groups C and W135.

In conclusion, we observed poor immune responses to both the group C and W135 portions of the PsACWY vaccine in those of <3 years of age, and we question the use of meningococcal polysaccharide vaccines (full or fractional dose) in this age group for the prevention of group C or W135 disease. Immunologic hyporesponsiveness was observed following a full dose of PsACWY vaccine at 12 to 23 months of age with subsequent vaccination with a 1/5 dose of PsACWY 10 months later compared to the response in PsACWY-naïve subjects receiving a 1/5 dose of PsACWY vaccine. The clinical relevance of immunologic hyporesponsiveness is unknown, but due to the rapid onset of meningococcal disease, circulating antibody is thought to be crucial in the protection against disease.

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