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Evaluation of De-O-Acetylated Meningococcal C Polysaccharide-Tetanus Toxoid Conjugate Vaccine in Infancy: Reactogenicity, Immunogenicity, Immunologic Priming, and Bactericidal Activity against O-Acetylated and De-O-Acetylated Serogroup C Strains

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The polysaccharide capsule of serogroup C *Neisseria meningitidis* (MenC) has been integral to vaccine development. Licensed MenC vaccines contain the O-acetylated (OAc+) form of polysaccharide. Some MenC strains have de-O-acetylated (OAc−) polysaccharide, which may affect antibody specificity and functional activity when used in a vaccine. We evaluated an OAc-MenC conjugate-tetanus toxoid conjugate (MCC-TT) vaccine given concomitantly with whole-cell diphtheria-tetanus-pertussis, *Haemophilus influenzae* type b, and oral polio immunization in 83 infants at 2, 3, and 4 months of age. Serum bactericidal activities (SBA) against OAc+ and OAc− MenC strains and OAc+ and OAc− polysaccharide-specific immunoglobulin G (IgG) levels were evaluated. MCC-TT vaccine was well tolerated. All infants produced SBA titers of ≥8 after a single dose at 2 months of age. The SBA geometric mean titer for OAc+ strain C11 increased from 2.7 (95% confidence interval [CI] 2.2 to 3.2) to 320 (95% CI, 237 to 432), 773 (95% CI, 609 to 982), and 1,063 (95% CI, 856 to 1319) after one, two, and three doses of MCC-TT, respectively. OAc− IgG levels were twice as high as OAc+ IgG levels after the primary series of MCC-TT vaccine, and the SBA was significantly higher against the OAc− MenC strain. Antibody responses to booster vaccination with either OAc+ MenC polysaccharide vaccine (MACP) or a fourth dose of MCC-TT at 14 months of age provided evidence of immunologic memory. The acetylation status of the booster vaccine influenced the specificity of the response, with significantly higher OAc− IgG levels and SBA after MCC-TT vaccine compared to MACP vaccine but similar OAc+ antibody levels. MCC-TT vaccine is highly immunogenic and primes for immunologic memory against OAc+ and OAc− MenC strains in infancy.

Serogroup C meningococcal (MenC) disease is an important cause of invasive bacterial infections in children and young adults in Europe and North America and is associated with significant mortality (25, 29). MenC polysaccharide vaccines are not effective in infants, who are at highest risk of disease (32). *Haemophilus influenzae* type b (Hib) conjugate vaccines provide long-term protection in young children and have virtually eliminated invasive Hib infections in developed countries (28). This technology has led to the development of MenC conjugate (MCC) vaccines that are immunogenic and prime for immunologic memory in infants and young children (18, 19, 26). The carrier protein used in conjugate vaccines may affect immunogenicity (15) and antibody responses to concomitant vaccines with the same carrier protein (8).

MenC polysaccharide (MCPS) is an α2→9 linked *N*-acetylneuraminic acid homopolymer with O-acetyl (OAc) groups at C-7 or C-8 residues. Some MenC strains (~12% of invasive isolates) produce a polysaccharide that lacks this OAc group (2, 6). The presence or absence of OAc groups generates unique epitopes, and the specificity of antibody binding to MCPS may affect its bactericidal activity against O-acetylated (OAc+) and de-O-acetylated (OAc−) strains (2, 21, 30). Licensed MCPS vaccines used in outbreak control in North America and Europe contain OAc+ polysaccharide. OAc+ MCC vaccines have been introduced in the United Kingdom and appear effective in infants and adolescents (1). Whether widespread use of these vaccines will favor the emergence of OAc− MenC strains is unknown.

Early studies found OAc− MCPS to be more immunogenic than OAc+ MCPS in adults and children (14, 31) but not in infants (24). North American Vaccine Inc. (Columbia, Md.) has developed a MenC conjugate vaccine containing OAc− MCPS conjugated to tetanus toxoid carrier protein (MCC-TT). It is well tolerated and immunogenic in adults, producing high levels of bactericidal antibody after a single dose (27). We evaluated the reactogenicity, immunogenicity, and immunologic priming of MCC-TT vaccine given at 2, 3, and 4

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TABLE 1. MenC-specific SBA titers to three serogroup C strains after MCC-TT vaccine administration at 2, 3 and 4 months of age and boosting with MCC-TT vaccine or MACP at 12 to 14 months of age

Sample timing	SBA GMT to C11 strain[a] (95% CI, *n*)	No. (%) of infant sera with C11 SBA of:			No. (%) of infants with ≥ 4-fold rise in SBA titer/total no. (%)[b]	SBA GMT to L91 543 strain[a] (95% CI, *n*)	SBA GMT to OAc+ C2a strain[a] (95% CI, *n*)
		≥8	≥32	≥128			
Before dose 1	2.6 (2.2–3.2, 77)	6 (8)	2 (3)	2 (3)	NA[c]	6.2 (4.4–8.8, 64)	2.5 (2.1–3.0, 77)
After dose 1	320 (237–432, 71)	71 (100)	68 (96)	63 (89)	65/69 (96)	1,192 (836–1,700, 64)	221 (166–295, 71)
After dose 2	773 (609–982, 79)	79 (100)	78 (99)	76 (96)	71/74 (96)	1,561 (1,227–1,987, 69)	499 (390–638, 79)
After dose 3	1,063 (856–1,319, 75)	75 (100)	75 (100)	75 (100)	68/69 (98)	2,114 (1,504–2,969, 44)	627 (498–791, 75)
At 12–14 mo	8.8 (5.9–13.1, 66)	31 (47)	18 (27)	8 (12)	NA	39 (23–66, 49)	6.9 (4.8–9.8, 66)
Post-MCC-TT booster	1,575 (903–2,748, 37)	37 (100)	37 (100)	35 (95)	36/37 (97)	8,579 (5,282–13,936, 30)	1,235 (748–2,039, 37)
Post-MACP booster	1,713 (945–3,103, 31)	31 (100)	31 (100)	29 (94)	29/31 (93)	3,336 (2,202–5,052, 27)	1,369 (746–2,515, 31)

[a] L91 543 serogroup C strain (C:2a:P1.5) is de-O-acetylated; the C11 strain (C:16:P1.7a,1) and C2a strain [M97.250926 (C:2a:P1.5,2)] are both O-acetylated.
[b] Fold rise in SBAs calculated in comparison to preimmunization titers at 2 months of age. Not all subjects had paired sera for each comparison; subjects receiving MCC-TT vaccine not achieving a fourfold rise had high SBA titers at 2 months.
[c] NA, not applicable.

months of age with routine infant immunizations. Antibody levels against OAc+ and OAc− MCPS, bactericidal activity against OAc+ and OAc− MenC strains, and antibody responses to concomitant vaccines containing TT were examined.

(This data was presented in part at the 12th International Pathogenic Neisseria Conference, Galveston, Tex., in November 2000.)

MATERIALS AND METHODS

Study population. Eighty-three healthy infants aged 7 to 10 weeks, eligible for immunization with whole-cell diphtheria-tetanus-pertussis (DTP), Hib, and oral polio vaccination were recruited between June and October 1997 from general practices in west Gloucestershire. Following informed written parental consent, the subjects were immunized at 2, 3 and 4 months of age, according to the schedule used in the United Kingdom. The West Gloucestershire local research ethics committee approved this study.

Vaccines and immunization. The MCC-TT vaccine consisted of 10 μg of OAc− MCPS coupled to approximately 15.5 μg of tetanus toxoid with aluminum hydroxide adjuvant (0.5 mg) in each 0.5-ml dose and 0.01% thimerosal as preservative. MCC-TT was given by intramuscular injection into the right anterolateral thigh, using a 25-gauge needle. At the same time, the infants received a 0.5-ml intramuscular injection of DTP vaccine (Trivax-AD; Wellcome, Manchester, United Kingdom) mixed with Hib-tetanus toxoid conjugate vaccine (Hiberix; SmithKline Beecham, Rixensart, Belgium) in the left thigh and oral polio vaccine. At 12 to 14 months of age, the children were randomized to receive either a 0.1-ml dose of meningococcal polysaccharide vaccine (MACP) [Mengivac (A+C); Pasteur Merieux, Lyon, France] containing 10 μg each of meningococcal A and C polysaccharides or a fourth dose (0.5 ml) of MCC-TT vaccine at the same time as measles-mumps-rubella vaccine (MMR-II; Pasteur Merieux). Reactogenicity was documented by carrying out parental interviews and using 7-day parental diaries recording axillary temperatures, local reactions, and systemic symptoms. Significant illnesses and hospitalizations during the study were documented. Blood samples were obtained prior to and 4 to 6 weeks after each immunization. Sera were separated, stored at −80°C, and transported frozen to the Public Health Laboratory Service Meningococcal Reference Unit, Manchester, United Kingdom, for analysis.

Serological studies. Sera were tested using standardized complement-mediated serum bactericidal assays against three MenC strains described previously (7, 26). The complement source was pooled baby rabbit serum (Pelfreeze Biologicals). Serum bactericidal activity (SBA) titers were expressed as the reciprocal of the final serum dilution giving ≥50% killing at 60 min. The strains used were the OAc+ C11 strain (phenotype C:16:P1.7a,1) and two clinical isolates representing the prevalent epidemic strains in the United Kingdom: OAc+ M97.250926 (C:2a:P1.5,2) and OAc− L91 543 (C:2a:P1.5). SBA titers of <4 were assigned a value of 2 for analysis. The serogroup C-specific immunoglobulin G (IgG) level was measured by standardized enzyme-linked immunosorbent assay (11) using the Centers for Disease Control and Prevention 1992 reference serum and OAc+ polysaccharide (code 98/730; supplied by NIBSC, Potters Bar, United Kingdom) and OAc− polysaccharide (supplied by the Centre for Applied Microbiology and Research, Porton Down, United Kingdom). The lower limit of the assay was 0.1 μg/ml, and sera with undetectable antibody levels were assigned a value of 0.05 μg/ml. Post-third-dose sera were also tested for IgG antibodies to tetanus toxoid and polyribosylribitol phosphate (PRP) using standardized assays as described previously (4, 12).

Statistical evaluation. Analysis was by intention to treat. Antibody levels were log-transformed, and geometric mean titers (GMT) and concentrations (GMC), with 95% confidence intervals, were calculated. Paired *t* tests were used to evaluate significance in differences between pre- and postvaccination antibody levels and between assays at each time point. Fisher's exact test was used to determine the significance of differences in the frequency of symptoms between vaccines. Student's *t* test was used to compare antibody levels between MCC and MACP booster vaccine recipients.

RESULTS

A total of 82 infants (43 male, 39 female) received three doses of MCC-TT vaccine with routine immunizations. One subject was withdrawn from the study at parental request after two doses. MCC-TT vaccine was well tolerated, with no serious adverse events related to vaccination and significantly less local reactions than those associated with the concurrent DTP-Hib immunization. Local erythema and swelling of ≥2.5 cm at the MCC-TT injection site occurred in 0.4 and 0.9% of children, respectively, compared to 4.8 and 10.2% after DTP-Hib immunization ($P < 0.003$ for both). Fever of ≥38°C was reported in 2.4% of infants within 3 days of vaccination. The rate of systemic reactions was similar to that in infants recruited from the same general practices who received DTP-Hib alone (12). Forty children received a fourth dose of MCC-TT vaccine, and 35 children received a dose of MACP vaccine at a median age of 57 weeks. Both booster vaccines were well tolerated, with no vaccine-related serious adverse events.

Immunogenicity. (i) SBA titers. MenC-specific SBA titers against the three strains are shown in Table 1. The SBA titers were low at 2 months of age, with most infants having no bactericidal antibody. MCC-TT vaccine was highly immunogenic after a single dose, with all infants having bactericidal antibody against all strains (100% SBA, ≥1:8) and 96% achieving a ≥4-fold rise in SBA titer against C11 strain (mean, 123-fold rise). Further significant increases in the C11 SBA GMT occurred after the second ($P < 0.001$) and third ($P = 0.002$) doses of MCC, with a mean 2.4-fold and 1.4-fold rise, respectively. Compared to the C11 SBA GMT, the GMT was lower for the OAc+ C:2a strain and higher for the OAc− strain after each dose ($P < 0.001$ for both). Insufficient

TABLE 2. MenC-specific IgG antibody levels according to O-acetylation specificity after MCC-TT vaccine administration at 2, 3, and 4 months of age and boosting with MCC-TT vaccine or MACP at 12 to 14 months of age

Sample timing	OAc− specific IgG GMC (95% CI, *n*)	No. (%) of infant sera with OAc− specific IgG of ≥2 μg/ml	OAc+ specific IgG GMC (95% CI, *n*)	No. (%) of infant sera with OAc+ specific IgG of ≥2 μg/ml
Before dose 1	0.35 (0.24–0.51, 60)	8 (13)	0.66 (0.5–0.86, 71)	12 (17)
After dose 1	38.0 (31.4–46.0, 64)	64 (100)	9.2 (7.4–11.3, 71)	69 (97)
After dose 2	48.97 (42.3–56.7, 68)	68 (100)	21.8 (18.7–25.4, 73)	73 (100)
After dose 3	51.7 (44.8–59.8, 62)	62 (100)	25.0 (21.1–29.5, 64)	64 (100)
At 12–14 mo	3.84 (3.0–4.8, 50)	40 (80)	3.63 (3.0–4.5, 54)	43 (80)
Post-MCC-TT booster	57.8 (45.0–74.2, 27)	27 (100)	40.96 (28.6–58.7, 28)	28 (100)
Post-MACP Booster[b]	36.56 (26.5–50.5, 25)	25 (100)	44.75 (31.7–63.2, 25)	25 (100)

amounts of some sera limited the number of assays performed; however, restriction of analysis to sera where all assays were performed did not affect the results (data not shown).

C11 SBA titers declined significantly by 12 months of age ($P < 0.001$), with only 47% of infants maintaining titers of ≥8. All 31 infants given a booster MACP vaccine had significant antibody responses, with a mean 170-fold rise in SBA titers against the C11 strain and 100% achieving titers of ≥32. A similar response was seen in infants boosted with MCC-TT vaccine, with a mean 218-fold rise in the C11 SBA titer. There was no significant difference in the SBA GMT to C11 and OAc+ C:2a strains between infants given MACP or MCC-TT boosters ($P = 0.83$ and 0.79, respectively). Infants given MCC-TT had significantly higher SBA GMT to the OAc− strain ($P = 0.004$) than did those receiving MACP vaccine.

(ii) Serogroup C-specific IgG antibody concentrations. IgG antibody concentrations in response to OAc+ and OAc− MCPS before and after each vaccination are shown in Table 2. At 2 months of age, antibody levels were low, and following a single dose of MCC-TT, there were significant rises in the IgG GMC for both OAc+ and OAc− MCPS ($P < 0.001$ for both). The levels were four times higher for the OAc− MCPS ($P < 0.001$). Smaller increases in IgG levels were seen after the second dose (OAc+, $P < 0.001$; OAc−, $P = 0.03$), and the increase in the IgG level after the third immunization was not significant for OAc+ ($P = 0.055$) or OAc− ($P = 0.2$) MCPS. The OAc− IgG GMC remained more than twice as high as the OAc+ GMC ($P < 0.001$). IgG levels fell by 12 months of age, although 80% of children had levels of ≥2 μg/ml, with no difference between OAc+ and OAc− IgG levels ($P = 0.58$). Significant rises in IgG levels occurred following both MACP and MCC-TT vaccination ($P < 0.001$). MCC-TT vaccine induced higher OAc− IgG than did MACP ($P = 0.020$) but induced similar levels of OAc+ IgG ($P = 0.72$), which was consistent with the pattern seen in SBA titer for OAc+ and OAc− strains.

(iii) Carrier protein responses. After three doses of DTP and PRP-T, the PRP-specific IgG GMC was 11.59 μg/ml (95% CI, 9.3 to 14.5; $n = 74$) and the tetanus antibody GMC was 4.00 IU/ml (95% Cl, 3.3 to 4.8). All children attained minimum protective antibody concentrations of 0.15 μg/ml for anti-PRP IgG and 0.01 IU/ml for tetanus.

DISCUSSION

Immunity to meningococcal disease correlates with the presence of bactericidal antibody against invasive meningococcal strains (14); however, the minimum protective SBA titer following vaccination is uncertain. In adults, a naturally acquired reciprocal SBA titer of 4, using a human complement source, was protective, but lower dilutions were not measured (13). The corresponding reciprocal SBA titer using a rabbit complement source has been estimated to be between 8 and 128. In a recent university outbreak of MenC disease, SBA titers obtained using rabbit complement were all <4 prior to or at the onset of invasive disease, suggesting that the presence of any bactericidal antibody is important, irrespective of the complement source used (17). MenC polysaccharide vaccines induce protective bactericidal anticapsular antibody in older children and adults (3, 20, 32), but young children produce low-avidity antibody that lacks bactericidal activity (16, 19, 20) and is not protective (32). The antibody response can be improved by conjugating MCPS to a carrier protein.

MCC-TT vaccine was well tolerated and highly immunogenic in infants on a 2-, 3-, and 4-month schedule. All infants produced bactericidal antibody after a single dose at 2 months of age, with higher seroconversion rates against the C11 strain than reported previously for other MCC vaccines given to infants from the same general practices using standardized assays in the same laboratory (10, 26). Further increases in antibody responses were seen after the second dose, but the response to a third dose was modest. This may result from the accelerated 2-, 3-, and 4-month schedule, and a higher response to a third dose may be seen under a 2-, 4-, and 6-month schedule. However, the SBA titers after one and two doses suggest that fewer than three doses are needed in infants, provided that there is adequate priming for immunological memory.

Antibody levels declined rapidly after primary immunization, as reported with other MCC vaccines (10, 26). The SBA response to the booster MCC-TT vaccine was higher than the response to primary immunization and the response in naive toddlers receiving MCC-TT vaccine at this age (27a). The antibody response to the booster MACP vaccine was greater than in naive children given this vaccine (7, 20, 22), confirming the successful induction of immunologic memory. The presence of memory is sufficient for long-term protection following administration of Hib conjugate vaccines (5, 28) and is expected to be sufficient after administration of MCC vaccines, although the high incidence of MenC disease in adolescence (25) means that a longer duration of protection is required. Theoretical concerns exist about the time to mount a protective antibody response, given the rapid onset of meningococcal

disease. The interval from initial carriage of the invasive strain to the onset of disease ranges from 2 days to 7 weeks in adults (9, 23) but is unknown in children. The kinetics of antibody responses at the mucosal level following initial carriage are poorly understood. Enhanced surveillance following the introduction of OAc+ MCC vaccines is proceeding to monitor any potential decline in efficacy with time and the need for booster doses since the British infant MCC immunization schedule does not include a scheduled booster (1).

MCC-TT induced twice as much IgG binding to OAc− MCPS compared to OAc+ MCPS, suggesting that ~50% of IgG recognized common backbone epitopes of MCPS and ~50% recognized unique OAc− epitopes created or exposed by the absence of the *O*-acetyl side chain. This influenced the functional activity against respective MenC strains, as documented previously (2, 21, 30), which may affect protection against carriage and invasive disease. The circulation of invasive OAc− strains may increase with the widespread use of OAc+ MCC (or MCPS) vaccines, and individuals with antibodies directed primarily against the unique epitopes created by the *O*-acetyl group in OAc+ MCPS may lack protective bactericidal antibody against OAc− strains. MCC-TT vaccine induced high levels of bactericidal antibody against both OAc+ and OAc− strains. The functional epitopes of MCPS are not clearly defined; however, bactericidal antibody appears directed predominantly against backbone epitopes in OAc+ and OAc− MCPS (21). OAc− MCPS is a more effective competitive inhibitor of bactericidal activity in human sera obtained after OAc+ MACP vaccination than after OAc+ MCPS (21). The presence of the OAc group may mask a protective epitope in the MCPS of the organism. The use of OAc− MCPS in a MCC vaccine may enhance its immunogenicity.

The type of MCPS used in booster vaccines influenced the specificity of the memory response. MCC-TT induced more OAc− IgG, whereas MACP vaccine induced similar amounts of OAc+ and OAc− IgG. Both vaccines induced high levels of SBA against all strains tested. Thus, MCC-TT is able to prime memory B cells specific for common backbone epitopes of MCPS, and these produce high levels of bactericidal antibody against OAc+ and OAc− strains. The higher SBA titers against the OAc− strain after MACP vaccine compared to the titers against the OAc+ strain may reflect the increased susceptibility of the OAc− strain to antibody-dependent complement-mediated lysis due to the lack of OAc groups, exposing epitopes to the more functional backbone-specific antibodies.

There was no evidence of decreased antibody responses to concomitant vaccines containing TT. Anti-PRP and anti-tetanus antibody GMC were higher than those seen with DTP-Hib alone in British infants reported previously in studies using the same assays (12). The lack of interference with PRP-T seen with pneumococcal-tetanus toxoid conjugate vaccines (8) may relate to differences in the schedule, the overall dose of tetanus toxoid administered, or the vaccine formulations used.

MCC-TT vaccine is well tolerated and highly immunogenic in infants. It induces high levels of bactericidal antibody and primes for immunologic memory against both OAc+ and OAc− MenC strains. Further studies are under way to assess the adequacy of a one- or two-dose schedule in infancy.

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