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Persistence of Group C Anticapsular Antibodies Two to Three Years After Immunization With an Investigational Quadrivalent *Neisseria meningitidis*-Diphtheria Toxoid Conjugate Vaccine

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Abstract

Background: An investigational quadrivalent (A, C, Y and W-135) meningococcal conjugate (MC-4) vaccine was reported to be more immunogenic in 2-year-olds than the currently licensed meningococcal polysaccharide vaccine, but persistence of serum antibody beyond 6 months after conjugate vaccination is unknown.

Objective: Determine persistence and the immunologic basis of protective activity of group C anticapsular antibodies in sera obtained 2–3 years after MC-4 vaccination.

Design: Group C antibody concentrations, bactericidal activity and passive protective activity were measured in sera from 48 children, ages 4–5 years, who had been immunized 2–3 years earlier with an MC-4 vaccine and from 47 children who had not been previously vaccinated.

Results: Serum antibody concentrations were higher in the vaccinated than the unvaccinated children (geometric means, 0.30 and 0.09 $\mu\text{g/mL}$, respectively, $P < 0.0001$). Bactericidal titers $\geq 1/4$ (considered protective) were infrequent in both vaccinated and unvaccinated children (14.6 and 6.4%, respectively, $P = 0.3$). Passive protective activity against bacteremia in the infant rat model was more frequent in sera from vaccinated (37.5%) than sera from unvaccinated children (12.5%, $P < 0.02$). The proportion of sera with passive protective activity increased with increasing anticapsular antibody concentrations ($P < 0.0001$).

Interpretation: Serum group C antibody concentrations remained elevated for 2–3 years after MC-4 vaccination, and passive protective activity was more frequent in vaccinated than unvaccinated children. However, serum antibody concentrations in many vaccinated children were no longer sufficient to activate complement-mediated bacteriolysis in vitro or to confer passive protection against experimental group C disease.

Keywords

Neisseria meningitidis; animal model; conjugate vaccine

An investigational quadrivalent meningococcal polysaccharide-diphtheria toxoid conjugate (MC-4) vaccine is being developed for prevention of disease caused by strains with capsular groups A, C, Y or W-135.^{1,2} We recently reported group C antibody responses of 2-year-olds immunized with this vaccine.³ In that study, children given a single dose of the investigational conjugate vaccine had significantly higher bactericidal antibody titers 1 and 6 months after vaccination than did 3-year-old control children who had been given a dose of a U.S.-licensed quadrivalent meningococcal polysaccharide vaccine. The group C serum antibodies elicited

by the conjugate vaccine also had higher antibody avidity and greater passive protective activity against group C bacteremia in the infant rat model than did serum group C anticapsular antibodies elicited by the polysaccharide vaccine.

In the present study, we investigated the duration and immunologic basis of protective activity of anticapsular antibodies in sera obtained 2–3 years after a dose of MC-4 vaccine given to children at 2–3 years of age. As in our previous study,³ we examined group C antibodies because group C strains cause a disproportionate number of deaths from meningococcal disease, as compared with strains with other capsular groups.⁴ Also the group C antigen in the currently licensed meningococcal polysaccharide vaccine is poorly immunogenic in 2- to 3-year-olds,^{5,6} and our laboratory established an infant rat challenge model to measure the ability of passively administered serum antibodies to confer protection against group C meningococcal bacteremia.^{3,5,7} A complete description of the serum antibody concentrations for all children enrolled in this follow-up study, as measured against all 4 capsular groups, and antibody responses to a challenge dose of quadrivalent meningococcal polysaccharide vaccine (to assess immunologic memory), will be reported separately.

MATERIALS AND METHODS

The original trial compared the safety and immunogenicity of an investigational MC-4 vaccine (Aventis Pasteur, Swiftwater, PA) with that of a U.S.-licensed meningococcal quadrivalent polysaccharide vaccine in 1398 children ages 2–10 years.⁸ In an extension of this trial, 102 children who had been immunized 2–3 years earlier with the MC-4 vaccine were seen in follow-up and provided serum samples. For the present study, sera from 48 of the children were chosen based on age of enrollment at the time of the extension study (4–5 years) and availability of sufficient volumes of sera for the studies described below. The mean age ($\pm$ SD) of this group was 2.6 ± 0.5 years at immunization, and 5.0 ± 0.5 years at follow-up. The mean interval ($\pm$ SD) between immunization and follow-up was 2.3 ± 0.3 years. As a control, we assayed sera from 47 healthy children ages 4–5 years (mean $\pm$ SD, 5.0 ± 0.5 years) who were cared for in the same pediatric practices where the study was conducted and who were not previously immunized with a meningococcal vaccine.

Concentrations of total serum group C anticapsular antibody (IgM, IgG and IgA) and antibody avidity constants, K_a , were measured by a radioantigen binding assay performed as previously described.⁵ Complement-mediated bactericidal titers were measured against group C strain 4243 with human complement. The assay was performed as previously described⁵ except that the buffer used was Dulbecco phosphate-buffered saline with 0.49 mM $MgCl_2$ and 0.79 mM $CaCl_2$ (Mediatech, Inc., Herndon, VA) and 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) instead of Gey's buffer with bovine serum albumin. Passive protective activity was measured in 5- to 7-day-old infant rats challenged intraperitoneally (ip) with group C strain 4243.^{3,5} At time 0, 100 μ L of a 1/4 dilution of serum were administered ip (2–3 rats/serum sample). Two hours later, the animals were challenged ip with 100 μ L containing $\sim$ 1000 colony-forming units (CFU) of washed, log phase *Neisseria meningitidis* group C bacteria. Blood specimens were obtained 18 hours after challenge. Dilutions of blood were plated onto chocolate agar and the CFU/mL was determined after overnight incubation of the plates at 37°C in 5% CO_2 . A protective serum was defined by a >2 log decrease in CFU/mL of blood in ≥ 2 rats, compared with the geometric mean CFU/mL of a group of negative control animals. Other statistical methods used for calculating geometric means, 95% confidence intervals, the probability for group differences and sensitivity and specificity are described in “Results” and in detail in our previous studies.^{9,10}

RESULTS

Figure 1A shows the respective reverse cumulative distributions of serum group C anticapsular antibody concentrations in children who were received the MC-4 vaccine 2–3 years earlier and in children of similar ages who were not previously vaccinated. On average, the anticapsular antibody concentrations in sera obtained from the vaccinated children were 3.3-fold higher than those from the unvaccinated children (geometric mean, 0.30 $\mu\text{g/mL}$ versus 0.09 $\mu\text{g/mL}$, respectively, $P < 0.0001$ by t test). The proportion of vaccinated children with serum anticapsular antibody concentrations of $>0.5 \mu\text{g/mL}$ (29.2%) was higher than that of unvaccinated children (6.3%, $P < 0.01$). There was no significant difference when the data were stratified at the $>1 \mu\text{g/mL}$ threshold (10.4 and 6.3%, respectively, $P > 0.5$).

Serum bactericidal titers of $\geq 1/4$ (considered protective¹¹) were infrequent in both previously vaccinated and unvaccinated children (14.6 and 6.4%, respectively, $P = 0.3$ by Fisher's exact test). Passive protective activity against group C meningococcal bacteremia in the infant rat challenge model was more frequent in sera from previously vaccinated children (18 of 48, 37.5%) compared with unvaccinated children (6 of 47, 12.8%, $P < 0.02$ by χ^2). As shown in Figure 1B, sera from the vaccinated children who had evidence of passive protective activity had, on average, 6.3-fold higher group C anticapsular antibody concentrations than did sera from vaccinated children who lacked passive protective activity (geometric means, 0.95 $\mu\text{g/mL}$ versus 0.15 $\mu\text{g/mL}$, respectively, $P < 0.0001$). A similar trend was observed between the concentrations of naturally acquired group C anticapsular antibody concentrations in protective and non-protective sera from the unvaccinated children (geometric means, 0.66 and 0.07 $\mu\text{g/mL}$, respectively, $P = 0.06$).

As summarized in Table 1, the proportion of sera with passive protective activity increased with increasing anticapsular antibody concentrations ($P < 0.0001$). Among the 15 sera with antibody concentrations between 0.31 and 0.99 $\mu\text{g/mL}$, a trend was observed toward higher antibody avidity in individuals with passive protective activity compared with those who that lacked protective activity. This difference was not significant (mean avidity of 28.9 nM^{-1} versus 22.0 nM^{-1} , respectively, $P = 0.14$). Regardless of the avidity constant or vaccinated or unvaccinated status of the child, a threshold antibody concentration of $>0.5 \mu\text{g/mL}$ was 63% sensitive and 99% specific for passive protective activity (Table 2). The results were similar if only data from vaccinated children were included in the analysis (67% sensitivity and 97% specificity).

Passive protective activity was 4.2-fold more common in sera with bactericidal titers of $\geq 1/4$ (8 of 10, 80%) than in sera with titers $< 1/4$ (16 of 85, 19%, $P < 0.0001$ by χ^2). However, the majority of the sera with passive protective activity (16 of 24, 67%) had bactericidal titers $< 1/4$. Therefore a bactericidal titer $\geq 1/4$ was specific (97%) for protective activity but lacked sensitivity (33%) (Table 2). This analysis included sera from both vaccinated and unvaccinated children. If only sera from the vaccinated children were included, the respective specificity (97%) and sensitivity (33%) were identical.

DISCUSSION

We investigated the persistence of serum group C anticapsular antibody 2–3 years after immunization of children with an investigational quadrivalent meningococcal polysaccharide protein conjugate vaccine. In the original trial, group C anticapsular antibody concentrations of 2-year-olds immunized with the MC vaccine peaked 1 month after vaccination (geometric mean, 3.1 $\mu\text{g/mL}$) and declined by 6 months (geometric mean, 0.69 $\mu\text{g/mL}$).³ In part because of avidity maturation between 1 and 6 months, the proportion of children with serum bactericidal titers of $\geq 1/4$ remained largely unchanged during the initial 6 months of follow-up (50% at 1 month and 40% at 6 months).³ Data from the present study show that by 2–3

years after vaccination, serum anticapsular antibody concentrations have declined further by ~2.3-fold compared with values seen at 6 months. Loss of bactericidal activity was also evident, given that only 14.6% of the sera from the vaccinated children had titers $\geq 1/4$. Similar results were reported in children given a single dose of a U.K.-licensed monovalent group C meningococcal conjugate vaccine at 1 year of age; serum antibody bactericidal titers had declined to baseline values by age 4 years.¹²

The infant rat model permits investigation of the protective activity of antibodies in an in vivo setting where the organism is rapidly replicating and is likely to be expressing different surface structures than organisms grown in artificial media.⁹ Passive protective activity against bacteremia in this model was present in 37.5% of sera from children given the MC-4 vaccine 2–3 years earlier. Although protection can be mediated by both anti-capsular or anti-noncapsular antibodies,^{7,10,13,14} the majority of the protective activity in the sera from the vaccinated children appears to have been mediated by group C anticapsular antibodies. The proportion of sera with protective activity increased with increasing group C anticapsular antibody concentrations ($P < 0.0001$). In addition, passive protective activity was 3-fold more frequent in sera from children vaccinated with the MC-4 vaccine than in sera obtained from unvaccinated children, which were used as a control ($P < 0.02$).

Serum bactericidal activity has long been considered a reliable immunologic surrogate of protection against meningococcal disease¹¹ (and reviewed in Ref. 15). However, absence of serum bactericidal activity is not necessarily associated with susceptibility to meningococcal disease because protection also can result from opsonic antibodies.¹⁶ This point was recognized by the authors of the original study reporting protection from group C disease in military recruits with serum bactericidal titers $\geq 1/4$.¹⁷ Furthermore recent experimental data⁹ from the infant rat challenge model demonstrate passive protective activity of naturally acquired group C anticapsular antibodies in some adult sera with bactericidal titers $< 1/4$ (measured with human complement), or $< 1/16$ (measured with rabbit complement, which increases the sensitivity of the assay^{17,18}). Data from the present study also demonstrate passive protective activity in some sera with bactericidal titers $< 1/4$ (human complement) obtained from children immunized for 2–3 years with MC-4 vaccine. In studies of naturally acquired group C anticapsular antibody in adults and conjugate-induced anticapsular antibody in children, a threshold serum anticapsular antibody concentration of ≥ 0.5 $\mu\text{g/mL}$ appears to be sufficient to confer passive protective activity regardless of the presence or absence of serum bactericidal activity. The most likely mechanism accounting for protective activity in the absence of serum bactericidal activity is opsonization mediated by antibody binding to the bacterial surface and activation of C3b deposition on the bacterial surface.^{13,14} Also in the animal model, the sera were diluted 1/4 and a dose of 100 μL was administered to the infant rats. Thus at a serum anticapsular antibody concentration of 0.5 $\mu\text{g/mL}$, the dose of antibody per rat required for protection was 0.0125 μg , a dose similar to that (0.008 μg of antibody per rat)³ used in our previous study of passive protection activity 6 months after MC-4 vaccination.

Meningococcal conjugate vaccines prime for immunologic memory, which is typically demonstrated by the ability of vaccinated children to develop IgG booster antibody responses to a challenge dose of unconjugated meningococcal polysaccharide vaccine.^{19,24} Induction of memory also can be inferred indirectly from serologic data showing avidity maturation.^{25,27} In a previous study, we demonstrated avidity maturation of group C serum anticapsular antibodies in sera obtained between 1 and 6 months after MC-4 vaccination.³ In contrast, avidity maturation was not detected in sera from control children immunized with unconjugated meningococcal polysaccharide vaccine.³ In other studies, evidence of memory B cells responsive to group C polysaccharide was observed in children 4–5 years after receiving a single dose of meningococcal conjugate vaccine.^{23,28} Thus we can infer that the vaccinated children in our study likely had immunologic memory to group C polysaccharide at the 2- to

3-year follow-up, which suggests that even those children with low serum anticapsular antibody concentrations could develop a booster responses if they encountered *N. meningitidis* group C bacteria. Recent evidence, however, suggests that immunologic memory alone may not be sufficient to confer protection against developing meningococcal disease.²⁹

In 1999, group C meningococcal conjugate vaccines were introduced in the United Kingdom and have been highly effective in eliminating group C disease.³⁰ At 4 years of follow-up, overall vaccine effectiveness remained at 90%.²⁹ However, a significant trend for waning overall effectiveness was observed beginning 1 year after vaccination. This trend was particularly evident in infants immunized using the accelerated U.K. schedule (2, 3 and 4 months of age) that does not include a booster injection in the second year (vaccine effectiveness of 93% within 1 year of vaccination but no efficacy in the period beyond 1 year²⁹). This schedule elicits high serum bactericidal antibody responses, which peak at 5 months of age and wane by 1 year.¹⁷ The schedule also primes for immunologic memory, which persists for at least 4 years.²⁸ Given the loss of vaccine effectiveness beyond 1 year in the immunized infants, the concept that immunologic memory alone is sufficient for long term protection is now in doubt, and alternative vaccination schedules are under consideration in the United Kingdom, including the addition of a booster dose late in infancy or in the second year of life. The U.K. data do not rule out the possibility that low concentrations of serum anticapsular antibody that are insufficient for activating complement-mediated bactericidal activity may be sufficient for conferring protection, a hypothesis that is consistent with passive protection data obtained in the infant rat model and the observed trend in the United Kingdom for group C conjugate vaccine effectiveness beyond 1 year after vaccination of toddlers, despite low or undetectable serum bactericidal titers.

In the present study, children vaccinated at 2–3 years of age with 1 dose of the MC-4 vaccine had higher serum group C anticapsular antibody concentrations than did unvaccinated children when measured in sera obtained 2–3 years after vaccination. A higher proportion of the sera from the vaccinated children also conferred passive protective activity in infant rats challenged with *N. meningitidis* group C. However, the serum antibody concentrations of the majority of the vaccinated children were no longer sufficient to activate complement-mediated bacteriolysis in vitro or to confer passive protection against experimental group C disease. Further data will be required to determine the safety and immunogenicity of a booster dose of the MC-4 vaccine in this age group, and the need and timing of a booster injection.

The serum samples used in this study were provided by Aventis Pasteur and were obtained by many physicians and nurses who conducted the vaccine trial at multiple clinical sites. Patricia Zuno Mitchell performed the serologic assays.

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FIGURE 1.

A, Reverse cumulative distributions of anticapsular antibody concentrations in sera obtained 2–3 years after MC-4 vaccination administered at a mean age $\pm$ SD of 2.6 ± 0.5 years ($N = 48$; —) and from unvaccinated children ($N = 47$; - - - -). B, Anticapsular antibody concentrations in relation to passive protective activity against group C meningococcal bacteremia in the infant rat model. Each circle represents the antibody concentration of an individual child. ●, Sera with passive protective activity (P); ○, sera that were not protective (NP). Horizontal bars represent the geometric mean antibody concentrations of the different groups.

TABLE 1

Relation of Serum Anticapsular Antibody Concentrations and Passive Protective Activity in the Infant Rat Challenge Model*

Anticapsular Antibody ($\mu\text{g/mL}$)	No. of Sera Tested	No. With Passive Protection
<0.3	72	6 (8.3) [†]
0.31–0.50	7	3 (42.9)
0.51–0.99	8	7 (87.5)
>1.0	8	8 (100)

* $P < 0.0001$ for trend (by χ^2). Data are combined for vaccinated and unvaccinated subjects ($N = 95$). Passive protection was tested in sera diluted 1/4. Protective activity was defined as a >2 log decrease in CFU/mL in blood cultures of ≥ 2 rats, as compared with the geometric mean CFU/mL of a group of rats pretreated with a 1/4 dilution of a negative control serum.

[†] Numbers in parentheses, percent.

TABLE 2

Sensitivity and Specificity of Serum Bactericidal Activity or Different Concentrations of Group C Anticapsular Antibody for Passive Protective Activity in the Infant Rat Bacteremia Model*

	Passive Protective Activity (%)	
	Sensitivity	Specificity
Serum bactericidal titer $\geq 1/4$	33 (16, 55)	97 (90, 100)
Anticapsular antibody ($\mu\text{g/mL}$)		
>0.3	78 (56, 93)	93 (84, 98)
>0.5	63 (41, 81)	99 (92, 100)
>1.0	33 (16, 53)	100 (95, 100)

* Passive protective activity in the rat model was considered “true positive” indicator of immunity to group C meningococcal disease. Data were combined for vaccinated and unvaccinated subjects (N = 95). Sensitivity (%) was defined as (number of sera with positive serology/number of protective sera) $\times$ 100. Specificity (%) was defined as (number of sera with negative serology/number of sera that lacked protective activity) $\times$ 100. The 95% confidence interval (numbers in parentheses) was calculated from the binomial distribution. When the respective sensitivities and specificities were calculated only for sera from the vaccinated children, the results were similar (see text).