

Effectiveness Analyses May Underestimate Protection of Infants after Group C Meningococcal Immunization

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Background. Group C meningococcal conjugate-vaccine effectiveness in the United Kingdom declines from ~90% in the first year to 0% between 1 and 4 years after immunization in infants immunized at 2, 3, and 4 months of age and to 61% in toddlers given a single dose. Confidence intervals are wide, and the extent of protection is uncertain.

Methods. Serum samples were obtained from children 3–5 years of age who were participants in a preschool booster-vaccine trial. Serum bactericidal activity was measured with human complement. Group C anticapsular antibody concentrations were measured by a radioantigen binding assay. Passive protection was analyzed in an infant rat bacteremia model.

Results. Serum samples from UK children who had been immunized 2–3 years earlier as infants or toddlers had higher levels of radioantigen binding, bactericidal activity, and passive protection than did historical control serum samples from unimmunized children ($P < .05$). A higher proportion of children immunized as infants had serum bactericidal activity titers $\geq 1:4$ (considered to be protective) than those immunized as toddlers (61% vs. 24%; $P < .01$), but there were no significant differences in the proportion of serum samples conferring passive protection (50% and 41%, respectively; $P = .4$).

Conclusions. We found no evidence of lower immunity in children immunized as infants than as toddlers. On the basis of serum bactericidal activity and/or passive protection, 40%–50% of both age groups are protected at 2–3 years after immunization, which was significantly greater than in unimmunized historical controls ($<5\%$).

In November 1999, the United Kingdom began routine immunization with monovalent group C meningococcal polysaccharide–protein conjugate vaccines. Immunization initially was offered to infants and teenagers, was then offered to all persons <18 years of age (~14 million), and was subsequently extended to persons <24

years of age. The immunization campaign was well received, with coverage reaching 85%–90% of children <18 years of age [1, 2]. Within 12–18 months after immunization was introduced, confirmed cases of group C disease in the age groups targeted for immunization fell by $>81\%$ [1], and group C carriage declined by 66% in teenagers [3]. Subsequently, there were also substantial declines in cases of group C disease in unimmunized persons of all ages, which was attributed to herd immunity [4].

The group C meningococcal-immunization campaign has been regarded as a major public health success [2]. However, in infants given 3 doses of conjugate vaccine, at 2, 3, and 4 months of age (the accelerated UK 3-dose immunization schedule), vaccine effectiveness (estimated using the screening method) declined from 93% in the first year to essentially 0% between 1 and 4 years after immunization [2]. Vaccine effectiveness in toddlers given 1 dose as part of the catch-up

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campaign also declined, from 88% in the first year to 61% between 1 and 4 years after immunization. These data have been interpreted as suggesting that vaccine effectiveness between 1 and 4 years after immunization is greater in toddlers given 1 dose of vaccine than in infants given the 3-dose accelerated schedule [2]. However, postlicensure estimates of vaccine effectiveness can be influenced by unintended bias. Also, the point estimates in the UK study were imprecise, with wide confidence intervals (CIs) resulting from the small number of cases in infants and toddlers [2].

Considerable data indicate that serum anticapsular antibodies confer protection against meningococcal disease (reviewed in [5]). Therefore, if group C meningococcal–vaccine effectiveness between 1 and 4 years after immunization is higher in those who were immunized as toddlers than in those who were immunized as infants, we would expect greater persistence of meningococcal antibody in serum samples from immunized toddlers. To test this hypothesis, we measured meningococcal antibodies in serum samples obtained from UK children who were immunized with group C meningococcal conjugate vaccines 2–3 years earlier as infants or toddlers. As a control, similar assays were performed on serum samples that had been obtained from age-comparable UK children before the introduction of meningococcal immunization.

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from children aged 3–5 years who were participants in a preschool booster-vaccine trial conducted in 2001–2002 by the Oxford Vaccine Group, University of Oxford [6]. On the basis of the availability of sufficient volumes, 105 serum samples were selected for studies of meningococcal immunity. Thirty-eight serum samples were from children who had been immunized with a group C conjugate vaccine at 2, 3, and 4 months of age as part of routine meningococcal immunization (i.e., “immunized infants”), and 67 serum samples were from children who had been immunized with a single dose of a group C conjugate vaccine given at age 1–2 years as part of the catch-up schedule (i.e., “immunized toddlers”). All of the immunized infants received 3 doses. Although information on the manufacturer of the group C conjugate vaccine used is not available, during the period when the immunization took place, nearly all infants and toddlers received the group C polysaccharide–CRM₁₉₇ conjugate vaccine produced by Wyeth Lederle Vaccines and Pediatrics (Meningitec). Stored serum samples from 31 children who had participated in a *Haemophilus influenzae* type b–immunization study conducted in Oxfordshire from 1994 to 1995, before the introduction of meningococcal immunization, served as “unimmunized historic controls.” Use of the serum samples for the present investigation was approved by the Oxfordshire Re-

search Ethics Committee and by the Institutional Review Board of Children’s Hospital and Research Center Oakland.

Serologic analysis. All assays were performed in a blinded manner, without knowledge of the age group or immunization status. Serum bactericidal activity was measured using log-phase, washed bacteria (strain 4243; C:2a:P1.5,2; ET37 complex and sequence type 11). The bacteria were grown for 2 h in Mueller-Hinton broth supplemented with 0.25% glucose (wt/vol) [7, 8]. After reaching an OD₆₂₀ of ~0.6, the culture was rediluted 1:7 into fresh Mueller-Hinton broth supplemented with 0.25% glucose (wt/vol) and 0.02 mmol/L cytidine-5′-monophospho-N-acetylneuraminic acid (Sigma-Aldrich) and incubated for an additional 2.0–2.5 h to an OD₆₂₀ of ~0.6. Except as noted, all test serum samples were heated at 56°C for 30 min to inactivate internal complement. The extrinsic complement source was serum from a healthy adult with a group C anticapsular antibody concentration of <0.06 µg/mL, as measured by a radioantigen binding assay (RABA) performed as described elsewhere [7], and with no detectable intrinsic bactericidal activity when serum concentration was tested at 20% or 40%. The bactericidal titer was defined as the dilution of serum resulting in a 50% decrease in colony-forming units per milliliter, compared with the colony-forming units per milliliter at time 0 in negative control samples. Concentrations of total (IgM, IgG, and IgA) serum anticapsular antibody to group C polysaccharide were measured by RABA as described elsewhere [7].

Passive protection. The passive protection assay has been described elsewhere [8]. In brief, 5- to 7-day-old pups from litters of outbred Wistar rats (Charles River) were randomly redistributed to the nursing mothers. At time 0, 100 µL of a 1:4 dilution of serum was administered intraperitoneally (ip) (*n* = 3–6 rats/serum sample). After 2 h, the rats were challenged ip with 100 µL of washed, log-phase bacteria (strain 4243). The challenge dose in different experiments ranged from 2200 to 5200 cfu/rat. Blood specimens were obtained 18 h after the bacterial challenge. Aliquots equivalent to 1, 10, and 100 µL of blood were plated onto chocolate agar. Protection was defined as a ≥95% decrease in the geometric mean number of colony-forming units per milliliter in the 3–6 rats given the test serum, compared with that in a group of negative control rats (average of ~460,000 cfu/mL in blood obtained 18 h after bacterial challenge).

Statistical analysis. For analyses of geometric means, samples below the lower limits of detection were assigned values of half of the lower limit (i.e., 1:2 for the bactericidal titer and 0.03 µg/mL for anticapsular antibody concentration measured by RABA). The respective geometric means of the antibody concentrations or titers and associated 2-sided 95% CIs were computed from the log₁₀ values. The proportion of immunized

Table 1. Age distribution of the Oxford study groups.

Study group	Subjects, no.	Age, ^a mean ± SD, years	Interval, ^b mean ± SD, years
Children immunized as infants at 2, 3, and 4 months (3 doses)	38	3.3 ± 0.13	2.9 ± 0.13
Children immunized as toddlers at 12–24 months (1 dose)	67	4.1 ± 0.31	1.9 ± 0.27
Unimmunized historic controls	31	3.6 ± 0.03	NA

NOTE. NA, not applicable.

^a At time of obtaining serum sample.

^b Between last immunization and time of serum sample collection.

subjects with serum bactericidal titers $\geq 1:4$ (considered a protective titer for group C) was computed. Differences found in the proportion of subjects in the groups with titers $\geq 1:4$ were analyzed by χ^2 or Fisher's exact test.

RESULTS

Demographics. The age distributions of the study groups at the time of obtaining the serum samples and the respective intervals between group C conjugate immunization and obtaining the serum samples are summarized in table 1. As expected, the follow-up period was ~ 1 year longer in the immunized infants than in the immunized toddlers because the children in the respective groups were of similar ages at the time of collection of the follow-up blood sample.

Anticapsular antibody responses. Figure 1A shows the reverse cumulative distribution of the total group C anticapsular antibody concentrations (IgM, IgG, and IgA) in serum samples obtained 2–3 years after the third dose of group C meningococcal conjugate vaccine in infants and after a single dose in toddlers, compared with those in unimmunized historic controls. The geometric mean serum antibody concentrations were higher in immunized infants or immunized toddlers than in unimmunized historic controls (0.82 and 0.56 $\mu\text{g/mL}$ vs. 0.08 $\mu\text{g/mL}$, respectively; $P < .0001$). There was a trend toward higher anticapsular antibody concentrations in immunized infants than there was in immunized toddlers ($P = .08$).

Serum bactericidal antibody titers. Figure 1B shows the reverse cumulative distribution of serum bactericidal titers in the immunized groups. The data for the unimmunized historic control serum samples are not shown, because none of the unimmunized historic control serum samples had a bactericidal titer $\geq 1:4$. For any given serum bactericidal titer, there was a greater percentage of serum samples from immunized infants that had this titer than from immunized toddlers. For example, the proportion of immunized infants who had bactericidal titers $\geq 1:4$ (considered to be protective when measured with human complement [9]) was higher than that of immunized toddlers (61% vs. 24%; $P < .01$). Even if the threshold for protection is considered to be a serum bactericidal titer $\geq 1:8$, then the re-

spective percentages of serum samples with protective titers is 50% for immunized infants, compared with 16% for the immunized toddlers ($P = .0006$). The geometric mean titer also was higher in the immunized infants (1:9 vs. 1:3 in toddlers; $P < .0001$).

Passive protective activity. All serum samples from the unimmunized historic control group ($n = 31$) and the immunized infant group ($n = 38$) and 66 of 67 serum samples from the immunized toddler group were tested at a 1:4 dilution for passive protection against group C meningococcal bacteremia in the infant rat model. As summarized in table 2, 50% of serum samples from immunized infants and 41% of serum samples from immunized toddlers conferred protection against group C bacteremia, compared with 3% of serum samples from unimmunized historic controls ($P < .0001$; χ^2). The mean decreases (expressed as \log_{10}) in colony-forming units per milliliter in the rats that received serum samples from immunized infants or immunized toddlers (compared with those in negative control rats) were 1.3–1.8 log greater than that of rats pretreated with serum samples from the unimmunized historical controls ($P < .0001$). There was no difference in the mean \log_{10} decrease in colony-forming units per milliliter between rats pretreated with serum from immunized infants and those pretreated with serum from immunized toddlers ($P = .17$).

Figure 2 summarizes serum passive protective activity stratified by bactericidal titers of $\geq 1:4$ or $< 1:4$ (figure 2A) or by total group C anticapsular antibody concentrations (figure 2B). Data are shown only for the immunized groups, because there were no bactericidal-positive serum samples from unimmunized historical controls to permit this analysis. As expected, the majority of serum samples with bactericidal titers $\geq 1:4$ from children in both immunized age groups conferred protection against group C bacteremia. Among children with bactericidal titers $< 1:4$, 30% of serum samples from children immunized as toddlers conferred protection, compared with 7% of immunized infants ($P = .09$; Fisher's exact test). This trend for greater protective activity in the bactericidal-negative serum samples of the immunized toddlers may explain the lack of an overall significant difference in passive protective activity in the

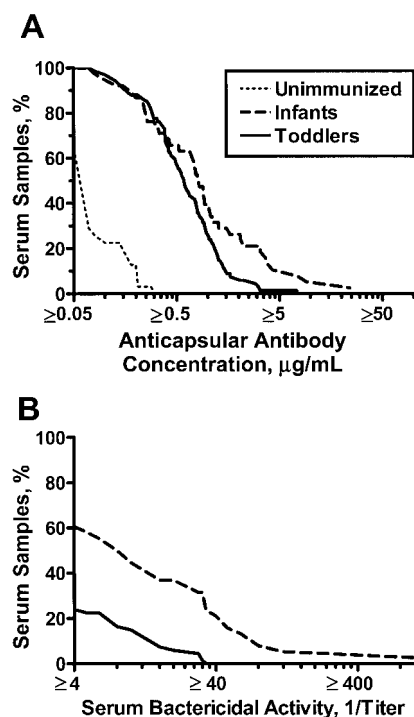


Figure 1. A, Reverse cumulative distribution of serum group C anticapsular antibody concentrations 2–3 years after group C meningococcal conjugate immunization. For comparison, the cumulative distribution of serum antibody concentrations of unimmunized historic controls is shown. Both immunized groups have higher geometric mean concentrations than the unimmunized control subjects ($P < .0001$). There is a trend toward higher geometric mean antibody concentrations in the children immunized as infants than in those immunized as toddlers ($P = .08$). This difference appears to be more pronounced at higher antibody concentrations. B, Reverse cumulative distribution of serum bactericidal titers for the 2 immunized groups. Data for the unimmunized historic control group are omitted, because none of these serum samples had bactericidal titers $\geq 1:4$. A higher percentage of serum samples from children immunized as infants had titers $\geq 1:4$, compared with serum samples from children immunized as toddlers (61% vs. 24%; $P < .01$). In addition, the geometric mean titer was higher in the children immunized as infants than in those immunized as toddlers (1:9 vs. 1:3; $P < .0001$).

2 immunized groups, because a greater percentage of the immunized infants had serum bactericidal titers $\geq 1:4$ (figure 1B). The proportion of serum samples conferring passive protection in the rat model increased with increasing serum anticapsular antibody concentrations (figure 2B). The respective trends for protective activity in the 2 immunized groups were not significantly different.

Serum samples from a group of children from South London who were immunized as infants. Because of the unexpected high prevalence of serum bactericidal activity in the cohort of children from Oxford immunized as infants, we assayed bactericidal activity in serum samples from a second group of 64 children, residing in South London, who had been participants in a *H. influenzae* type b–booster-vaccine trial conducted in

2003 (PTH, unpublished data). Their mean \pm SD age (years) at the time of collection of the serum samples was 2.8 ± 0.5 . These children were immunized with a group C meningococcal conjugate vaccine 2.5 years earlier, by use of the routine 3-dose accelerated UK infant immunization schedule. The prevalence of group C bactericidal titers of $\geq 1:4$ in this serum sample collection was 35% (95% CI, 23%–47%), compared with 24% (95% CI, 14%–36%) in the serum samples from the immunized toddlers from Oxford. Unfortunately, the serum samples from the children from South London had inadvertently been heated at 65°C for 30 min to inactivate internal complement activity, instead of at the correct temperature of 56°C. In experiments with 22 pre- or postimmunization serum samples from adults, heating at 65°C for 30 min instead of 56°C decreased the prevalence of group C meningococcal bactericidal titers of $\geq 1:4$ by 21% and the geometric mean titer by 30%. Thus, given the excessive heat treatment of the collection of serum samples from children in South London, the 35% prevalence of bactericidal activity is a minimum estimate of the titers present ~ 2.5 years after group C immunization of infants.

Compatibility of the serologic data with surveillance data.

As described above, vaccine effectiveness 1–4 years after immunization of infants in the United Kingdom has been estimated to be 0% (on the basis of the occurrence of 18 cases of group C disease in immunized children vs. 1 in unimmunized children) [2]. To assess the compatibility between the previously published estimate of vaccine effectiveness [2] and different levels of vaccine effectiveness, we estimated underlying coverage levels from the published data and then calculated, by exact binomial calculation under the assumptions of vaccine effectiveness of 30%, 40%, 50%, and 60%, the probability of observing results as extreme as those observed in the published data. When vaccine coverage of 91% is assumed, the probability that at least 18 cases would have been in immunized children is 0.29 for a true vaccine effectiveness of 30%, 0.22 for an effectiveness of 40%, 0.15 for an effectiveness of 50%, and 0.08 for an effectiveness of 60%. Even if the true coverage was as low as 89% or as high as 93%, the observed result would still have had a 9% or 27% probability of occurring, respectively, if the true vaccine effectiveness was 50%.

DISCUSSION

The United Kingdom was the first country to introduce routine immunization with group C meningococcal conjugate vaccines. After initiation of the immunization campaign, the number of cases of group C meningococcal disease decreased dramatically in both immunized and unimmunized individuals (the decline in the latter was attributed to herd immunity [4]). The decrease in the number of cases was associated with a decrease in group C carriage [3]. During the first year after immunization, vaccine effectiveness was estimated to be high in all age groups: for

Table 2. Passive protection against bacteremia in infant rats challenged with *Neisseria meningitidis* group C.

Study group	Children tested, no.	Children protected, % (95% CI) ^a	Mean log ₁₀ decrease in bacterial count, cfu/mL ^b
Children immunized as infants at 2, 3, and 4 months (3 doses)	38	50 (33–67) ^c	1.8 ^d
Children immunized as toddlers at 12–24 months (1 dose)	66	41 (15–54) ^c	1.3 ^d
Unimmunized historic controls	31	3 (0–17)	0.1

NOTE. CI, confidence interval.

^a Serum samples were tested at a dilution of 1:4 (3–6 rats/serum sample). Protective serum was defined as that giving a 95% decrease in geometric mean no. of colony-forming units per milliliter in blood cultures obtained 18 h after bacterial challenge, compared with rats treated with negative control serum that gave no protection. $P < .0001$, χ^2 analysis (2 *df*).

^b Compared with the geometric mean no. of colony-forming units per milliliter in rats given the negative control serum. $P < .0001$; analysis of variance.

^c $P = .4$; Fisher's exact test, comparing immunized groups.

^d $P = .17$; *t* test, comparing immunized groups.

example, it was 93% in infants given the 3-dose accelerated immunization schedule and 88% and 96%, respectively, in children 1–2 years of age and adolescents 11–16 years of age who were given 1 dose of vaccine during the catch-up campaign [2]. However, vaccine effectiveness dropped between 1 and 4 years after immunization, to essentially 0% in immunized infants and to 61% in immunized toddlers, but it remained high in immunized teenagers (90%). As noted above, the point estimates of vaccine effectiveness in the immunized infants or immunized toddlers are imprecise, because of the small numbers of cases and corresponding wide 95% CIs (upper limits of 94% in toddlers and 71% in infants). Therefore, although vaccine effectiveness between 1 and 4 years is thought to be higher in children given a single dose as toddlers than in infants given the 3-dose accelerated schedule, it is not known whether there are true differences in effectiveness between the 2 immunization schedules.

In the present study, we evaluated persistence of serum group C antibodies 2–3 years after routine immunization of infants given the 3-dose schedule and of toddlers given the catch-up 1-dose schedule. Both immunized cohorts had higher serum

bactericidal titers, higher anticapsular antibody concentrations, and greater serum passive protective activity against group C bacteremia in the infant rat model than unimmunized naive historical control subjects had. Furthermore, in contrast to the reported higher vaccine effectiveness 1–4 years after immunization in immunized toddlers than in immunized infants, we found a higher prevalence of serum bactericidal activity ($P < .01$) and a trend for higher serum group C anticapsular antibody concentrations in the immunized infants ($P = .08$). Prevalence of passive protective activity was also at least as high in the serum samples from the immunized infants (50%) as in the serum samples from immunized toddlers (42%) ($P = .4$). Thus, on the basis of 3 different serologic assays, we can infer that vaccine effectiveness 2–3 years after immunization is either higher or not inferior in children given the accelerated 3-dose infant immunization schedule than in children given the 1-dose toddler catch-up schedule. However, we cannot exclude the possibility that the proportion of immunized toddlers with serum bactericidal titers $<1:4$ who are protected against meningococcal disease is higher than that of immunized infants with serum bactericidal titers $<1:4$, as is suggested by the ob-

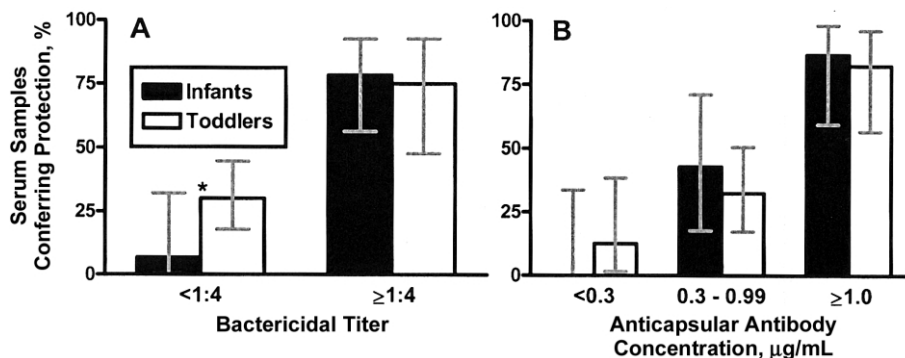


Figure 2. Passive protection of infant rats against group C meningococcal bacteremia in relation to serum antibody. *A*, Bactericidal activity. *B*, Anticapsular antibody. Error bars represent 95% confidence intervals (CIs). The asterisk indicates that the probability of the observed difference in protective activity in the groups with titers $<1:4$ is $P = .09$.

served trends in passive protective activity in the infant rat model (figure 2A).

The serum samples assayed in the present study were obtained from children living in the Oxford region who were enrolled in a preschool booster-vaccine study conducted in 2001–2002 [6]. Thus, one important limitation of the present study is that we cannot be certain that the serum antibody levels measured are representative of those in children living in other areas of the United Kingdom. However, the seroprevalence of group C meningococcal bactericidal antibody in a second set of serum samples, from children residing in South London who were immunized as infants, was compatible with the results from Oxford. Therefore, the data from Oxford are not unique to that region. Considering the data from the 2 regions together, we conclude that the prevalence of protective serum bactericidal titers 2–3 years after the UK 3-dose accelerated immunization schedule in infants is not inferior and may be higher than after 1 dose of vaccine given to toddlers. Further, given the large body of data indicating that serum bactericidal activity confers protection against meningococcal disease (reviewed in [10]), it seems unlikely that vaccine effectiveness between 1 and 4 years after the 3-dose UK accelerated infant immunization schedule is 0%, as was determined by analysis of surveillance data [2]. This conclusion is also supported by the observations in the infant rat bacteremia model, in which 50% of the serum samples from immunized infants had protective activity. The reason for the disparity between the estimate of vaccine effectiveness determined by epidemiologic surveillance and that inferred from seroprevalence data is unknown. However, given the small number of cases ascertained by surveillance, if true vaccine effectiveness between 1 and 4 years after immunization of infants is between 30% and 50%, the probability of finding no effectiveness by chance alone was high (0.29 and 0.15, respectively).

Several studies have shown that the serum antibody titers elicited in infants and young children by group C meningococcal conjugate vaccine wane over time [11–14]. However, immunologic priming persists for at least 4 years after immunization [15]. But, in contrast to expectations before licensure of the group C conjugate vaccines [10], priming for a memory antibody response in the absence of a protective serum antibody concentration does not appear to be sufficient to confer solid protection against meningococcal disease [2]. Currently, there is excellent control of group C meningococcal disease in the United Kingdom, in large part because of a high prevalence of protective serum antibody titers in immunized older children and young adults and a low probability that susceptible persons will encounter the pathogen, because of the effect that immunization has on decreasing group C carriage in the population [3, 4]. Over time, however, the likelihood of exposure to group C organisms may increase in the United Kingdom, and, therefore, children immunized as infants or toddlers may require a booster dose.

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