Disparity in Functional Activity between Serum Anticapsular Antibodies Induced in Adults by Immunization with an Investigational Group A and C *Neisseria meningitidis*-Diphtheria Toxoid Conjugate Vaccine and by a Polysaccharide Vaccine

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Polysaccharide-protein conjugate vaccines elicit higher concentrations of serum anticapsular antibody in infants and children than do unconjugated polysaccharide vaccines. The conjugate-induced antibodies also have higher avidity and complement-mediated bactericidal activity. Similar vaccine-related differences in the magnitude or functional activity of antibody are observed infrequently in immunized adults. We compared the antibody responses of adults immunized with an investigational group A and C meningococcal conjugate vaccine to those elicited by an unconjugated meningococcal polysaccharide vaccine. Although there were no significant differences between the respective geometric mean bactericidal titers of the two vaccine groups, it took, on average, three- to fourfold higher concentrations of polysaccharide-induced serum anticapsular antibody to achieve 50% complement-mediated bacteriolysis than conjugate-induced antibody ($P < 0.001$ for groups A and C). At limiting doses, the polysaccharide-induced anticapsular antibodies also were less effective in conferring passive protection against meningococcal bacteremia in infant rats challenged with a group C strain ($P < 0.04$). The avidity index of the group C antibodies was higher in the conjugate vaccine group than in the polysaccharide vaccine group ($P < 0.005$). The disparities in the functional activity of the anticapsular antibodies elicited in adults by the two vaccines imply fundamental differences in the respective B-cell populations stimulated.

Polysaccharide-protein conjugate vaccines are highly effective for the prevention of invasive diseases caused by a number of encapsulated bacteria, including *Haemophilus influenzae* type b (42), *Streptococcus pneumoniae* (seven serotypes) (13, 25), and *Neisseria meningitidis* group C (38). These vaccines are highly immunogenic in infants and young children, who typically show low serum antibody responses to unconjugated polysaccharide vaccines. The conjugate vaccines also prime for memory antibody responses to a subsequent exposure to the respective unconjugated polysaccharide (see, for example, references 6, 10, 19, 33, 40, and 45). Priming for such memory responses may be an important additional mechanism of protection in conjugate-immunized persons whose serum anticapsular antibody concentrations are below the protective threshold (reviewed in reference 29).

Polysaccharide-protein conjugate vaccines are also immunogenic in adults (2, 9, 15, 17, 24, 26, 30, 36, 37, 44, 46). With a few notable exceptions (12, 36), however, the available evidence suggests that immunization of adults (or elderly individuals) (37) with conjugate vaccines offers no appreciable advantages over immunization with the corresponding unconjugated polysaccharides with respect to both the magnitude of the serum antibody responses and, with one possible exception (18), the induction of immunologic memory (26, 37).

Multivalent meningococcal conjugate vaccines are under de-

velopment and likely will be licensed in Europe and the United States in the relatively near future (39). It is expected that these conjugate vaccines will be recommended for all age groups, including adults, and will replace the currently available tetravalent meningococcal polysaccharide vaccine. Although in adults unconjugated meningococcal polysaccharide vaccines elicit high concentrations of anticapsular antibody and are estimated to confer protection against disease for up to 5 to 10 years (1, 47), there are theoretical advantages of using conjugate vaccines in this age group. First, several studies of adults given meningococcal polysaccharide vaccine have shown that serum group C anticapsular antibody responses to subsequent doses of unconjugated meningococcal group A and C polysaccharide vaccines are lower than those to the initial dose (7, 8, 18, 26, 32, 41). Such hyporesponsiveness could increase the risk of acquiring meningococcal disease in an immunized person whose serum antibody concentrations had fallen below the protective threshold. Use of conjugate vaccines may avoid this problem. Second, it is possible that antibody avidity or complement-mediated bactericidal activity may be superior after immunization with conjugate than after immunization with unconjugated meningococcal vaccines, as seen in infants (11) and toddlers (20, 28, 33), although the limited available data addressing this question in adults do not show evidence of affinity maturation after conjugate immunization (15).

We compared here the bactericidal activity of serum anticapsular antibodies elicited in adults by an investigational group A and C meningococcal conjugate vaccine with that of a licensed bivalent A and C meningococcal polysaccharide vac-

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TABLE 1. Bactericidal titers of sera investigated in the present study compared to the entire sample in the original study^a

Vaccine received	Bactericidal antibody ^b (1/titer)			
	Subset ^b		Entire study ^c	
	Mean log $\pm$ SE	Geometric mean	Mean log $\pm$ SE	Geometric mean
Group A antibody				
Conjugate	3.75 $\pm$ 0.39	5,574	3.83 $\pm$ 0.04	6,693
Polysaccharide	3.89 $\pm$ 0.40	7,845	3.98 $\pm$ 0.04	9,654
Group C antibody				
Conjugate	3.39 $\pm$ 0.61	2,483	3.36 $\pm$ 0.06	2,299
Polysaccharide	3.54 $\pm$ 0.67	3,444	3.58 $\pm$ 0.06	3,805

^a All samples were assayed at Aventis Pasteur laboratories by the standardized CDC method with rabbit complement (35).

^b Eighteen adults received group A and C meningococcal conjugate vaccine, and 16 adults received the control meningococcal A and C polysaccharide vaccine.

^c Ninety-six adult sera were tested from the group receiving the group A and C meningococcal conjugate vaccine, and ninety-four adult sera were tested from the group receiving the control meningococcal A and C polysaccharide vaccine (26).

cine. We also compared the ability of the respective antibodies to confer protection against bacteremia in infant rats challenged with group C meningococcus.

MATERIALS AND METHODS

Serum samples. We utilized stored serum samples that had been collected as part of a previously published immunogenicity study conducted at the Sheffield Institute for Vaccine Studies, Sheffield Children's Hospital, in the United Kingdom (26, 48). In brief, 195 healthy adults, aged 18 to 30 years, were randomized to receive one dose of either a licensed meningococcal polysaccharide vaccine ($n = 95$ subjects; Meningivac; Aventis Pasteur-MSD, Maidenhead, United Kingdom) or an investigational bivalent group A+C meningococcal conjugate vaccine ($n = 100$ subjects) prepared by Aventis Pasteur. This conjugate vaccine had been previously tested in infants in Niger (11). Each dose of the investigational conjugate vaccine contained 4 μ g each of group A and C polysaccharides conjugated to 48 μ g of diphtheria toxoid protein. Each dose of Meningivac contained 50 μ g each of group A and C polysaccharides. Serum samples were collected 4 to 8 weeks after immunization and stored frozen at -70°C .

For the present study, we assayed a convenience sample of the stored sera selected based on availability of sufficient quantities. The sera were coded, and the vaccine assignments were disclosed only after all of the data had been reported to the sponsor, Aventis Pasteur (Swiftwater, Pa.). Eighteen of the sera were from adults immunized with conjugate vaccine, and 16 were from adults immunized with the control polysaccharide vaccine. As shown in Table 1, the respective geometric mean bactericidal titers of these sera measured at Aventis Pasteur by the Centers for Disease Control (CDC) standardized method with rabbit complement paralleled the results from all of the subjects in the original study. The Institutional Review Board of Children's Hospital Oakland and Research Center at Oakland approved the use of the sera for the present study.

RABA. The concentrations of groups A and C anticapsular antibody were measured by a radioantigen-binding assay (RABA), performed as previously described (16, 21). The group A meningococcal polysaccharide was derivatized with tyramine via CNBr and provided by Emil Gotschlich (The Rockefeller University). The group C meningococcal polysaccharide (provided by Aventis Pasteur) was derivatized in our laboratory with tyramine by a modification of the method of Lees et al. (27). Antibody concentrations in the test sera were determined by comparison of the percentage of binding of the radiolabeled antigen by different dilutions of the test sample to that of a standard curve of binding at different dilutions of an antimeningococcal group A/C human reference serum (CDC1992 [obtained from the National Institute for Biological Standards and Controls, Hertfordshire, United Kingdom]). This reference serum has been assigned a group A anticapsular antibody concentration of 135.8 $\mu\text{g/ml}$ and a group C antibody concentration of 32.0 $\mu\text{g/ml}$ (22).

ELISA. The concentrations of immunoglobulin G (IgG) antibody to groups A and C polysaccharides were measured by enzyme-linked immunosorbent assay

(ELISA). The group A assay was performed at Aventis Pasteur by using polysaccharide mixed with methylated human albumin as the target antigen (35). The group C assay was performed at CHORI by using polysaccharide derivatized with adipic dihydrazide as the target antigen, prepared as previously described (20). Antibody concentrations were assigned to the test sera by using the CDC1992 reference serum (IgG antibody concentrations of 91.8 $\mu\text{g/ml}$ for group A and of 24.1 $\mu\text{g/ml}$ for group C [22]).

Group C antibody avidity index. The avidity index was measured by using a modification of a previously described chaotropic elution ELISA (14, 15). Briefly, microtiter plates were coated with group C polysaccharide derivatized with adipic dihydrazide as described above for the group C ELISA procedure. Serum samples were diluted in 1% (wt/vol) bovine serum albumin–0.1% (wt/vol) Tween–phosphate-buffered saline to give an anticapsular antibody concentration yielding an optical density of ca. 1.0 in the ELISA. After overnight incubation at 4°C , plates were washed and incubated for 15 min at room temperature with various concentrations of ammonium thiocyanate (0 to 1 M). Plates were washed and developed as for the ELISA. The avidity index is the concentration of thiocyanate required to decrease the absorbance by 50%.

Complement-dependent bactericidal antibody activity. Bactericidal activity was measured by using log-phase bacteria that had been grown for ca. 2 to 2.5 h in Mueller-Hinton broth supplemented with 0.25% (wt/vol) glucose (43). Bactericidal activity was measured against group A meningococcal strain Z1073 (kindly provided by Mark Achtman, Max-Planck Institute, Berlin, Germany) and group C strain 4243 (kindly provided by Trudy V. Murphy [CDC, Atlanta, Ga.]). For the measurement of bactericidal activity, all test sera were heated at 57°C for 30 min to inactivate intrinsic complement activity. The extrinsic complement source was serum obtained from a healthy adult with no detectable intrinsic bactericidal activity against the target strains when tested at a final serum concentration of 40% (twofold higher than the complement concentration used to test bactericidal activity in the test sera).

Passive protection against bacteremia in an animal challenge model. The animal challenge model has been described previously (21). In brief, 4- to 7-day-old pups from litters of outbred Wistar rats (Charles River, Raleigh, N.C.) were randomly redistributed to the nursing mothers. At time zero, animals were treated intraperitoneally (i.p.) with 100 μl of test serum that had been diluted based on the RABA result to administer 0.04, 0.008, and 0.0016 μg of group C anticapsular antibody. Negative control rats were treated with a 1:5 dilution of sera from an unimmunized adult. For each serum, three animals were treated with each dose (a total of nine animals per serum). After 2 h the animals were challenged i.p. with 100 μl of log-phase bacteria from group C strain 4243 diluted to deliver 1,300 CFU/rat. Heparinized blood samples were obtained by cardiac puncture 18 h later, and aliquots of 100, 10, and 10 μl of a 1:10 dilution (i.e., 1 μl) of blood were plated onto chocolate agar. At each antibody dose, "protection" was defined as sterile cultures in at least two of the three rats tested. For calculation of the geometric mean CFU/milliliter of blood, animals with sterile cultures were assigned a value of 1 CFU/ml, and animals with a "lawn" of bacteria on culture plates streaked with 1 μl of blood were assumed to have $>500,000$ CFU/ml of blood and were assigned a value of 10^6 CFU/ml.

Statistical analysis. The 50% bactericidal concentration (BC_{50}) was calculated by dividing the anticapsular antibody concentration of each serum sample by the respective bactericidal titer. For calculation of group geometric means, the antibody concentrations or titers were logarithmically transformed (base_{10}). For log transformation, samples below the lower limits of detection were assigned values half of the lower limit (i.e., 1:2 for the bactericidal titer and 0.005 and 0.01 $\mu\text{g/ml}$, respectively, for group A and C anticapsular antibody by RABA and 0.02 $\mu\text{g/ml}$ for IgG group C anticapsular antibody by ELISA). In the passive protection assay, a geometric mean CFU/milliliter for each serum sample was assigned at each antibody dose tested, based on the quantitative culture results of the three treated animals. The geometric means of the CFU/milliliter for each vaccine group at each antibody dose tested were calculated from these data ($n = 10$ sera per vaccine group). Statistical differences between respective group geometric means were calculated by using a two-tailed Student t test (Excel software).

RESULTS

Table 2 summarizes the geometric means of the respective group A and C anticapsular antibody concentrations and bactericidal titers of adults assigned to each of the vaccine groups. Although not shown in Table 2, the geometric mean IgM anticapsular antibody concentrations of the conjugate group for capsule groups A and C were 1.2 and 0.9 $\mu\text{g/ml}$, and the

TABLE 2. Antibody levels in sera obtained 4 to 8 weeks after immunization of adults with meningococcal conjugate vaccine or meningococcal polysaccharide vaccine^a

Vaccine received	Anticapsular antibody ($\mu\text{g/ml}$)				Bactericidal antibody ^b (1/titer)		
	Total determined by RABA		IgG determined by ELISA		Mean log $\pm$ SE	Geometric mean	% with ≥ 8
	Mean log $\pm$ SE	Geometric mean	Mean log $\pm$ SE	Geometric mean			
Group A antibody							
Conjugate	1.39 $\pm$ 0.13	24.7	1.48 $\pm$ 0.17	30.5	2.05 $\pm$ 0.16	111	100
Polysaccharide	1.58 $\pm$ 0.14	38.4	1.65 $\pm$ 0.16	45.1	1.73 $\pm$ 0.16	54	94
Group C antibody							
Conjugate	1.14 $\pm$ 0.12 ^c	13.9	1.08 $\pm$ 0.18 ^c	12.1	1.83 $\pm$ 0.17	67	100
Polysaccharide	1.67 $\pm$ 0.14 ^c	46.9	1.61 $\pm$ 0.16 ^c	39.4	1.72 $\pm$ 0.18	53	94

^a Eighteen adults received group A + C meningococcal conjugate vaccine, and 16 adults received the control meningococcal A + C polysaccharide vaccine.

^b Bactericidal activity was measured against group A strain Z1073 and group C strain 4243 by using human complement.

^c The total and IgG group C anticapsular antibody concentrations are greater in adults receiving polysaccharide vaccine than in adults receiving conjugate vaccine ($P < 0.01$ for total antibody measured by RABA and $P < 0.04$ for IgG measured by ELISA). Other respective differences were not significant ($P > 0.10$).

corresponding values for the polysaccharide vaccine were 2.4 and 2.3 $\mu\text{g/ml}$. Thus, the antibody responses in both vaccine groups were predominantly IgG.

The geometric mean of the total A anticapsular antibody concentration was ca. 50% higher in the sera from adults immunized with the polysaccharide vaccine compared to that of the conjugate vaccine group, but the respective difference was not significant ($P > 0.10$). The respective IgG anticapsular responses of the two vaccine groups paralleled the total antibody responses. (Note that the slightly higher geometric mean IgG antibody concentrations measured by ELISA compared to the respective total antibody concentrations measured by RABA are within the expected range of variability of the two different methods). In contrast, there was an opposite trend in the respective group A bactericidal antibody responses, with higher bactericidal titers in the conjugate-vaccinated group than in the polysaccharide-vaccinated group, but the difference was not statistically significant (reciprocal geometric means of 111 versus 54 [$P > 0.10$]).

The geometric means of the total and IgG group C anticapsular antibody concentrations were significantly higher in the polysaccharide vaccine group than the conjugate-vaccinated

group ($P < 0.05$ for total and IgG). There was no significant difference, however, in the respective group C bactericidal antibody response (reciprocal geometric mean titer of 53 in the polysaccharide group versus 67 in the conjugate group [$P > 0.10$]).

Figure 1 shows graphically the concentrations of anticapsular antibody required for BC_{50} in the complement-mediated bactericidal assay as measured against group A strain Z1073 (Fig. 1A) and group C strain 4243 (Fig. 1B). For both capsular groups A and C, the antibody concentrations required to elicit 50% complement-dependent killing were significantly lower in the sera from the adults given the conjugate vaccine than those given the polysaccharide vaccine. For group A, the respective geometric mean BC_{50} values were 0.22 and 0.71 $\mu\text{g/ml}$ ($P \leq 0.001$ by both a t test and the nonparametric Mann-Whitney test); for group C, the respective values were 0.21 and 0.89 $\mu\text{g/ml}$ ($P < 0.001$ by both tests). Approximately one-quarter of the subjects in the conjugate vaccine group had BC_{50} values of ≤ 0.10 $\mu\text{g/ml}$, values that were not observed in the polysaccharide vaccine group, and approximately one-third of the polysaccharide vaccine group had values of ≥ 1.0 , which were not observed in the conjugate vaccine group. The BC_{50} values for

FIG. 1. BC_{50} in complement-mediated bactericidal assay. (A) BC_{50} measured against group A strain Z1073. (B) BC_{50} measured against group C strain 4243. Horizontal bars represent geometric mean concentrations. The geometric mean BC_{50} values of the adults given the conjugate vaccine are significantly lower than the corresponding values for the polysaccharide group ($P < 0.001$ for both groups A and C).

FIG. 2. Group C anticapsular antibody avidity index as measured by a thiocyanate elution ELISA. The geometric mean avidity index in adults given the conjugate vaccine is significantly greater than in adults given polysaccharide vaccine (330 versus 170 [$P < 0.005$]). The difference remained statistically significant after removal of the three outliers in the conjugate group ($P < 0.01$).

the remaining subjects in the two groups overlapped. The respective distributions were similar for capsular groups A and C. The different distributions of the BC_{50} values of the two vaccine groups were significantly different for both groups A and C ($P \leq 0.005$ by chi-square analysis).

The avidity index was measured for the group C antibodies by using a chaotropic elution ELISA as described in Materials and Methods. (The index was not measured for group A because of insufficient quantities of sera and the lack of an established method for group A antibody in our laboratory.) As shown in Fig. 2, the geometric mean avidity index of the group C anticapsular antibodies induced by the conjugate vaccine was significantly higher than that of the antibodies induced by the polysaccharide vaccine (330 versus 170 [$P < 0.005$]).

To determine whether the differences in vitro between the two vaccine groups in antibody avidity indices and BC_{50} concentrations might have biological relevance, we investigated the ability of 10 individual sera selected randomly from each vaccine group to confer passive protection against meningococcal bacteremia in infant rats challenged with group C strain 4243. As summarized in Table 3, at an antibody dose of 0.008 $\mu\text{g}/\text{rat}$ ca. 50% of the sera from each vaccine group showed protective activity. At a fivefold-lower dose (0.0016 $\mu\text{g}/\text{rat}$),

TABLE 3. Passive protection against bacteremia in infant rats challenged with *N. meningitidis* group C strain 4243^a

Antibody dose ($\mu\text{g}/\text{rat}$)	Conjugate vaccine		Polysaccharide vaccine	
	No. of protective sera/total no.	CFU/ml (geometric mean)	No. of protective sera/total no.	CFU/ml (geometric mean)
0.04	9/10	5	10/10	3
0.008	5/10	72	6/10	47
0.0016	2/10	12,900 ^c	0/10	168,000 ^c

^a Five-day-old rats were treated i.p. with antibody at time zero and then challenged 2 h later with 1,300 CFU of bacteria. Quantitative blood cultures were obtained 18 h after challenge. Rats treated with negative control sera from unimmunized adults had $>500,000$ CFU/ml of blood.

^b Each dose of antibody for an individual serum was tested in three rats. Serum was considered protective if at least two of three treated animals at each antibody dose had sterile cultures. A CFU/milliliter value was assigned for each serum at each antibody from the geometric mean CFU/milliliter of the triad.

^c $P < 0.04$ (Student *t* test).

however, 2 of 10 sera from the conjugate group had protective activity compared to 0 of 10 sera in the polysaccharide group ($P > 0.10$ by Fisher exact test). The geometric mean CFU/milliliter in blood from the animals pretreated with polysaccharide-induced antibody was more than 10-fold higher than that from animals pretreated with an identical dose of serum antibodies induced by the conjugate vaccine (168,000 versus 12,900 CFU/ml [$P < 0.04$]).

DISCUSSION

Unconjugated capsular polysaccharide vaccines elicit serum antibody responses largely in the absence of T-cell help (so-called T-cell-independent [TI] antigens) (reviewed in references 31 and 29). TI antigens are immunogenic in older children and adults but are typically poorly immunogenic in infants, and repeated injections do not elicit memory type antibody responses at any age. The development of protein-polysaccharide conjugate vaccines overcomes many of these limitations by presenting the polysaccharide covalently coupled to a carrier protein, which confers thymus-dependent (TD) properties on the polysaccharide and results in increased immunogenicity of the polysaccharide component in young children, higher antibody avidity, and higher complement-mediated bactericidal activity. The conjugate vaccine also primes for immunologic memory (reviewed in reference 29, 31).

The superior features of immune responses to polysaccharide conjugate vaccines are unambiguous in infants and young children. The data, however, in adults are less clear since adults typically respond to unconjugated capsular polysaccharide vaccines with high concentrations of anticapsular antibodies in serum that confer protection against disease. The ability of adults to respond well to polysaccharide vaccines has been considered to be an indicator of intrinsic B-cell maturation. Acquisition of antibody responsiveness, however, also may reflect natural priming and generation of anticapsular B-cell populations in the memory state since anticapsular antibodies elicited in adults by capsular polysaccharide vaccination show features of a secondary antibody response being isotype switched and hypermutated (3, 4, 23, 29, 49). It is not surprising, therefore, that most previous studies comparing the immunogenicity of meningococcal conjugate and unconjugated polysaccharide vaccines in adults failed to detect significantly higher serum anticapsular antibody concentrations (2, 15, 41) or bactericidal titers (2, 15, 41) in the conjugate-vaccinated group. One exception was a study in adolescents by Choo et al., who found higher group C bactericidal antibody responses in serum after immunization with a group C conjugate vaccine than after immunization with a polysaccharide vaccine (12). Of interest, the Choo study used human complement to measure the bactericidal titers, whereas the studies reporting no significant differences in bactericidal titers or higher titers after immunization with polysaccharide vaccine used rabbit complement. Compared to human complement, rabbit complement has been shown to augment meningococcal bactericidal titers substantially (5, 34, 43, 50), particularly in children given polysaccharide vaccine (43).

In the present study, we reassayed a subset of the sera from our previously reported study of adults immunized with a group A and group C conjugate vaccine (26). The respective

serum group A and C geometric mean anticapsular antibody concentrations measured by the RABA on the subset of sera from the two vaccine groups (Table 2) showed a similar distribution to the previously published IgG anticapsular antibody concentrations measured by ELISA for all of the subjects in this trial (26).

The published study, which used rabbit complement to measure bactericidal activity, reported significantly higher group A and C bactericidal titers in the polysaccharide-vaccinated group than in the conjugate-vaccinated group (Table 1, $P < 0.01$). Using human complement and different meningococcal test strains to measure bactericidal activity, we found opposing trends in the present study, with higher group A and C bactericidal titers in the conjugate-vaccinated adults, although the respective differences between the two vaccine groups were not statistically significant (Table 2). Based on the magnitude of the respective bactericidal antibody responses and the proportions of individual with protective titers of $\geq 1:8$ ($\geq 94\%$ of both vaccine groups), we would expect that both vaccines would be equally efficacious in conferring short-term protection against meningococcal disease.

The higher anticapsular antibody responses in the polysaccharide-vaccinated adults, and the opposing trends for lower bactericidal titers, suggested qualitative differences in the anticapsular antibody populations elicited by the two vaccines. Analyses of the BC_{50} s in the sera from subjects given the conjugate vaccine compared to those given the polysaccharide vaccine (Fig. 1) unequivocally confirmed this hypothesis. To elicit complement-mediated bacteriolysis of both group A and C strains, it took, on average, three- to fourfold-lower concentrations of anticapsular antibodies in serum from adults vaccinated with conjugate vaccine than from those vaccinated with polysaccharide vaccine (Fig. 1). The sera from the polysaccharide-vaccinated adults also showed a wider range of BC_{50} s than the sera from the conjugate vaccine group. The lower BC_{50} s in the conjugate vaccine group may have resulted from a higher-avidity anticapsular antibody, as demonstrated for group C (Fig. 2). Using a similar chaotropic ELISA, Goldblatt et al. found no significant differences in the geometric mean avidity index of antibodies in sera obtained from adults 1 month after vaccination with a group C meningococcal conjugate vaccine (prepared by Wyeth Lederle Vaccines) or meningococcal polysaccharide vaccine (15). The reason for the discrepant results between that study and the present study is unknown. Both studies employed the same polysaccharide vaccine as a control, but the different avidity results between the respective conjugate and polysaccharide vaccine groups could reflect the use of different conjugate vaccines in the two studies or different doses of the conjugate vaccines (4 μ g of each polysaccharide in the present study versus 10 μ g of group C polysaccharide in the study by Goldblatt et al.).

In the present study, the *in vivo* protection data in rats challenged with group C strain 4243 (Table 3) were consistent with our *in vitro* observations of superior functional activity of antibodies elicited in adults by the conjugate vaccine. At the lowest antibody dose tested, there was greater protection against bacteremia conferred by the conjugate-induced anticapsular antibodies than by antibodies elicited by the unconjugated polysaccharide vaccine. These data underscore that

vaccine-induced meningococcal anticapsular antibodies from immunized adults have variable protective activity and that, when serum antibody concentrations are low, such variation may be important in determining whether or not there is protection against disease.

In a previous study, we reported that fivefold-higher doses of anticapsular antibody were required to confer passive protection against group C bacteremia in infant rats pretreated with sera from children immunized with meningococcal polysaccharide vaccine at 4 to 5 years of age than in infant rats pretreated with sera from immunized adults (21). Further, pretreatment with similar doses of serum anticapsular antibody elicited by vaccination of 2- to 3-year-old children failed to confer protection. These data indicated age-related variations in the functional activity of group C anticapsular antibodies induced by meningococcal polysaccharide vaccine (21). The results of the present study demonstrate vaccine-related variation in antibody functional activity of groups A and C anticapsular antibodies elicited in adults by conjugate or polysaccharide vaccine. The molecular basis of these vaccine-related differences in antibody function will require further study. The higher functional activity of the conjugate-induced antibodies could reflect selection of different memory B-cell subsets accompanied by differential somatic hypermutation. Note also that the dose of vaccine may be critical. For example, the polysaccharide vaccine contained 50 μ g each of group A and C polysaccharides as opposed to 4 μ g each of group A and C polysaccharides in the conjugate vaccine. Higher polysaccharide doses could result in deletion of certain memory B-cell subsets or preferential stimulation of lower-avidity B cells.

Whatever the underlying mechanism, our results underscore the difficulties in defining an absolute "protective threshold concentration" of serum group A or C anticapsular antibody since the concentration of anticapsular antibody required to confer protection may vary considerably with the nature of the immunogen used (i.e., TD versus TI) and/or the age at immunization. Finally, the relationship between antibody titers detected and functional activity *in vivo* may differ between the different assay methods used for assessing antibody responses.

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