Human Opsonins to Meningococci After Vaccination

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Two groups of volunteers were immunized with either a serogroup A plus C meningococcal polysaccharide vaccine or a combined serogroup B polysaccharide-serotype 2 protein vaccine. Serum opsonin responses were measured by chemiluminescence of polymorphonuclear leukocytes exposed to opsonized live meningococci. Two of the six volunteers immunized with the A plus C vaccine had an increase in serum opsonins to group A meningococci, four responded to group C meningococci, and none to group B meningococci. Five other volunteers who were immunized with the combined group B polysaccharide-serotype 2 protein vaccine responded with an increase in serum opsonins to group B meningococci of two different protein serotypes, as well as to a group C-serotype 2 meningococcal strain. Although no booster effect was observed after a second dose of the combined vaccine, both the polysaccharide and the protein components appear to be able to stimulate an opsonin response.

An effective vaccine against group B meningococci is not yet available. In contrast to results of immunizations with the capsular polysaccharides from group A and C meningococci (12), vaccination of humans with the corresponding group B polysaccharide antigen has not led to a significant seroconversion rate as measured by bactericidal antibodies (18). Likewise, vaccines containing outer membrane proteins alone have not resulted in a satisfactory response (7, 20), although they are immunogenic in mice (2) and guinea pigs (8). Combined polysaccharide-protein vaccines, on the other hand, have initiated bactericidal antibodies in humans (4, 19, 21).

Since phagocytosis is of major importance in host defense against other encapsulated bacteria and since meningococci are phagocytized (15, 16), studies of serum opsonins may provide valuable information about resistance to meningococcal infection. We therefore measured opsonins to live meningococci in sera from human volunteers immunized with either a group A plus C meningococcal polysaccharide vaccine or a combined group B polysaccharide-serotype 2 protein vaccine. Serum opsonins were quantified by chemiluminescence production of polymorphonuclear leukocytes (PMNLs) during phagocytosis of meningococci which were opsonized with sera from the volunteers (13).

MATERIALS AND METHODS

Immunizations. Six healthy medical students (designated volunteers a through f) were given one subcutaneous dose (0.5 ml) of a commercially available vaccine against group A and C meningococci (Institut Mérieux, Lyon, France) containing 50 µg each of group A and C polysaccharides. Blood samples were drawn before, and 2, 7, and 60 weeks after immunization.

Five healthy individuals (designated volunteers g through k) among our laboratory personnel received a combined group B polysaccharide-serotype 2 protein vaccine (lot 790626VB), prepared as described previously (6). One dose of this vaccine contained 50 µg of group B polysaccharide, 50 µg of outer membrane protein (serotype 2), 8 µg of lipopolysaccharide, 50 µg of thimerosal, 3 mg of lactose, and 4.25 mg of NaCl dissolved in 0.5 ml of sterile water. Two doses were given as deep subcutaneous injections 4 weeks apart, and blood samples were drawn just before the first dose and 2 and 5 weeks thereafter. The sera were stored at −70°C until used.

Leukocytes. Human PMNLs from unvaccinated healthy adult volunteers were prepared from heparinized whole blood sedimented with dextran solution (60 g/liter) as described previously (13). The erythrocytes were hemolyzed with NH4Cl (8 g/liter), and the leukocytes were washed twice in phosphate-buffered saline (pH 7.2) before being suspended in Hank's balanced salt solution (HBSS) containing 5 g of bovine serum albumin per liter to make a final concentration of 107 PMNLs per ml.

Bacteria. Seven Neisseria meningitidis strains were used in the opsonization assay: clinical isolates of group A (17295/82), group C-type 2 (08141/81), group B-type 2 (60/78), and group B-type 15 (42442/80 and 32768/81), recovered from patients with meningitis or septicemia, as well as the group B-type 2 vaccine strain (M986) and a group C-type 2 strain (1381). The vaccine group B strain was used in the bactericidal assay. All strains were freeze dried or stored at −70°C in Grevé's solution (50 g of bovine serum albumin, 50 g of sodium glutamate, and 100 g of glycerol per liter). Before use the bacteria were cultured overnight on blood agar plates at 37°C in an air atmosphere with 10% CO2 and 80% relative humidity. Plate scrapings were inoculated into heart infusion broth containing 1 g of agar per liter and incubated at 37°C with continuous shaking. Viable bacteria were harvested in the log phase (2 h), washed in phosphate-buffered saline, and resuspended in HBSS containing 5 g of bovine serum albumin per liter. The suspensions were adjusted to an optical density of 1.0 at 620 nm and a 10-mm light path, which corresponded to 3.8 ± 1.6 × 108 CFU/ml.

Measurement of serum opsonins. Opsonins to the bacteria were measured by a chemiluminescence method (13). Briefly, 0.2 ml of the serum to be tested was added to 2.3 ml of HBSS mixed with 0.5 ml of the bacterial suspension. After 2 min at 20°C, 0.5 ml of the leukocyte suspension was added,
and chemiluminescence at 37°C was determined by reading counts per minute for periods of 30 s after 12 and 24 min, with a Beckman LS 1000 scintillation counter (Beckman Instruments, Inc., Irvine, Calif.) out of phase with one photomultiplier disconnected. Serum opsonic activity was expressed as counts per minute and was calculated by subtracting the counts obtained with samples containing HBSS instead of serum (12 × 10^3 to 30 × 10^3 cpm) from the counts of samples containing serum. The control samples incubated in HBSS gave readings from 20 to 35% of the maximum readings obtained with sera from those samples which gave the highest response. The readings obtained at 12 min (given in the figures) largely corresponded to those at 24 min.

The precision of the method at different levels of activity was good; the coefficients of variation on repeated testing of the same samples on the same day were less than 7%. However, the day-to-day variation with PMNLs from three different donors on 3 consecutive days was large (59%). Therefore, the figures presented for each volunteer and organism represent results obtained on the same day with the PMNLs from one donor.

**Bactericidal antibodies.** A microbacterial assay, based on a method described by Frasch et al. (8) with baby rabbit serum as complement source, was used to test the sera from the volunteers immunized with the group B vaccine. The group B-serotype 2 vaccine strain (M986) was used as the test organism.

**Statistical methods.** Student's t-test and the Wilcoxon signed rank test were used to evaluate significances of proportional differences.

**RESULTS**

Two of the six volunteers immunized with the A plus C vaccine had more than fourfold increases in serum opsonic activity against group A meningococci (Fig. 1). High serum levels persisted for more than a year after vaccination. Minor or no response was seen in the other four volunteers, two of whom had low preimmunization levels of opsonins against group A. Four of the six individuals immunized with the A plus C vaccine also had more than fourfold increases in opsonins towards group C meningococci, and a persistent response was seen in two of them (Fig. 2). Sera from the two individuals who had the highest preimmunization levels to group C gave no increased chemiluminescence response. Seven weeks after vaccination the increase in opsonic activity compared with preimmunization activity was significant by Student's t-test to both group A (P < 0.02) and group C (P < 0.05) meningococci. However, with the Wilcoxon signed rank test and allowing for a minimum of six individuals, the postimmunization increases were significant for neither group A (P = 0.05) nor group C (P > 0.10). The sera from the A plus C vaccinated individuals were also examined for opsonic activity against group B meningococci, and no individual showed increase in opsonins between pre- and 7 weeks postimmunization sera (data not shown).

The combined group B polysaccharide-outer membrane protein meningococcal vaccine induced an increase in opsonic activity towards the vaccine group B-serotype 2 strain (Fig. 3). Three of the five volunteers had more than fourfold increases, whereas the other two had more than twofold and threefold increases, respectively. The chemiluminescence response 2 and 5 weeks after immunization was significant (P < 0.01 with Student's t-test). In addition, a single experiment (due to there being no more sera left) showed a more pronounced response towards a group B-type 2 clinical isolate, confirming that a response to vaccination had occurred. In contrast, only two of five volunteers immunized with the combined polysaccharide-protein vaccine showed a fourfold or greater increase in bactericidal antibody titers against the group B-type 2 vaccine strain (Fig. 4). However, the preimmunization group B bactericidal antibody titers were 512 or greater in four of the five volunteers. The increases in bactericidal antibody titers were elicited in two of the individuals with the lowest preimmunization anti-group B antibodies. The group B vaccine also failed to induce a booster response against group B strains in any of

**FIG. 1.** Opsonins against a group A meningococcal clinical isolate in sera from six volunteers (designated a through f) immunized once (arrow) with a group A plus C polysaccharide vaccine. Serum opsonins are recorded in counts per minute as chemiluminescence responses of polymorphonuclear leukocytes 12 min after exposure to meningococci sensitized with sera from the volunteers.

**FIG. 2.** Opsonins against a group C meningococcal clinical isolate in sera from six volunteers (designated a through f) immunized once (arrow) with a group A plus C polysaccharide vaccine. Serum opsonins were measured by the chemiluminescence method and recorded as counts per minute as described in the legend to Fig. 1.
the five volunteers, as measured by either bactericidal antibodies or opsonins.

After immunization with the combined group B vaccine, more than twofold increases in opsonins to a group B-type 15 clinical isolate was demonstrated in all the volunteers \((P < 0.05)\) (Fig. 5). Moreover, sera from two volunteers (no more preimmunization serum left from the other three) also showed more than twofold opsonic responses against a group C-type 2 meningococcus. Control experiments with the group B vaccine strain at the same time as the group B-type 2 and -type 15 clinical isolates revealed much greater chemiluminescence responses with the clinical isolates than with the vaccine strain.

**DISCUSSION**

After vaccination with the group A plus C meningococcal polysaccharide vaccine, some of the volunteers showed a marked increase in serum opsonic activity against these groups of meningococci. Since they were immunized with pure group A plus C polysaccharides and experienced a rapid and long-lasting increase in groups A and C-specific opsonic activity, this activity probably represented antibodies to the capsular polysaccharides. Moreover, the levels of serum opsonins measured in their postimmunization sera were comparable to the activity found in sera from meningococcal disease convalescents (13).

The lack of an obvious opsonic response to group A meningococci in four of our volunteers is in contrast to the results of clinical trials showing an excellent protective effect against group A meningococcal disease (14). The marked increase we observed in opsonins against group C meningococci, on the other hand, corresponds with the good protective effect of vaccination against infections with these bacteria in adults (11). Most likely, high prevaccination levels of opsonins reflect previous contact with the bacterial antigen in question and may explain the lack of an increased opsonic response in some. This is also in accordance with previous observations in adults that polysaccharide antigens have poor ability to boost a bactericidal antibody response (10).

As expected from studies on bactericidal antibodies (12), we did not find evidence of cross-reacting opsonin response to group B meningococci after immunization with the A plus C polysaccharide vaccine.

Our study showed that the group B polysaccharide-protein vaccine induced increases in opsonic activity against the vaccine strain M986 in all five volunteers, but it induced increases in bactericidal antibody in only two of them. However, the test strain influenced the results of the group B chemiluminescence assay; when a corresponding clinical isolate (group B-serotype 2) was used as the test organism, the chemiluminescence responses were much higher. Cranen et al. (3) found the strain M986 to be rather resistant to killing by fresh normal human serum, and this might explain the relative resistance of this strain to opsonization.

The combined group B-type 2 vaccine induced a marked opsonic response against a group B-type 15 clinical isolate in all five volunteers. This may be due in part to stimulation of antibodies to the polysaccharide component of the vaccine. However, the vaccine also contains lipopolysaccharide and non-serotype 2 outer membrane proteins. These components might have stimulated an opsonin response to other group B strains (1). Since a response to a group C-serotype 2 meningococcal strain was demonstrated in some of the volunteers, and since there was no evidence of cross-reactivity between group C and group B polysaccharides, it is probable that type-specific antibodies are also involved in the opsonin response.

After immunization with the combined group B-serotype 2 vaccine, the bactericidal antibody titers to the vaccine strain increased in only two of the volunteers, those who had the lowest preexisting antibody levels. Although more consistent increases in bactericidal antibodies have been found in other studies (9), differences inherent in the techniques may also explain the discrepancies between our bactericidal assay results and the results of the opsonin assay (17). In our study serum opsonins were measured without exogenous complement, whereas baby rabbit complement was used in the testing for bactericidal antibodies. Zollinger et al. (19) demonstrated that human postvaccination antibodies to meningococcal group B polysaccharide were strongly bactericidal with rabbit complement but had little or no bactericidal activity in conjunction with human complement. Moreover, the antibodies that were bactericidal with human complement appeared to be primarily directed against noncapsular antigens (19).

Since results of studies with a chicken embryo model indicate that opsonizing antibodies to group B meningococci may be important in protection against group B disease (5), our technique for testing of opsonins may represent an
additional tool for measuring vaccine responses. The number of volunteers in our study should have been larger for an adequate statistical analysis to be performed. Further studies are in progress to determine the nature and specificity of the opsonins. It also remains to be seen whether the increases in opsonic activity in humans correspond with resistance to infection.

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LITERATURE CITED


