Effect of Vaccine-Elicited Antibodies on Colonization of Neisseria meningitidis Serogroup B and C Strains in a Human Bronchial Epithelial Cell Culture Model

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ABSTRACT Capsular polysaccharide-protein conjugate vaccines protect individuals from invasive disease and decrease carriage, which reduces spread of the organism in the population. In contrast, antibodies elicited by plain polysaccharide or protein antigen-based meningococcal (Men) vaccines have little or no effect on decreasing carriage. In this study, we investigated the mechanism by which vaccine-induced human immunoglobulin G (IgG) antibodies affect colonization by meningococcal serogroup B (MenB) or C (MenC) strains using a human bronchial epithelial cell culture model (16HBE14o-). Fluorescence microscopy showed that bacteria colonizing the apical side of 16HBE14o- monolayers had decreased capsular polysaccharide on the bacterial surface that resulted from shedding the capsule and not decreased production of polysaccharide. Capsular polysaccharide shedding depended on the presence of 16HBE14o- cells and bacteria but not direct adherence of the bacteria to the cells. Treatment of bacteria and cells with postimmunization MenC-conjugate IgG or murine anti-MenB polysaccharide monoclonal antibodies (MAbs) inhibited capsule shedding, microcolony dispersal, and invasion of the 16HBE14o- cell monolayer. In contrast, the IgG responses elicited by immunization with MenC polysaccharide (PS), MenB outer membrane vesicle (OMV)-based, or factor H binding protein (FHbp)-based vaccines were not different than preimmune IgG or no-treatment response. The results provide new insights on the mechanism by which high-avidity anticapsular antibodies elicited by polysaccharide-conjugate vaccines affect meningococcal colonization. The data also suggest that any effect on colonization by IgG elicited by OMV- or FHbp-based vaccines may involve a different mechanism.

KEYWORDS Neisseria meningitidis, capsular polysaccharide, colonization, vaccines

Neisseria meningitidis is a bacterial species that normally colonizes human upper airway epithelial cells. For reasons that are not completely understood (1), some strains move through the epithelial cell layer into the bloodstream, causing rapidly progressing bacteremia and meningitis, with relatively high rates of mortality and debilitating sequelae in survivors. Humans provide the only reservoir of meningococci, and transmission between individuals occurs through mucosal aerosols, with infants, children, and young adults having the highest rates of disease.

Like Haemophilus influenzae and Streptococcus pneumoniae, which can also cause bacteremia and meningitis, pathogenic meningococcal strains are encapsulated by polysaccharides. Strains producing meningococcal serogroup A, B, C, W, X, or Y capsular polysaccharides (MenABCWXYP S) cause most cases of meningococcal disease. Except for MenB PS, which is chemically similar to human polysialic acid, meningococcal capsular PSs are immunogenic, and vaccines containing them provide individual protection against disease (2). However, plain PS-based meningococcal vaccines have
little effect on colonization and provide little or no protection against disease in the
unvaccinated person through herd immunity. In contrast, as was first shown for
*Haemophilus influenzae* capsular PS-protein conjugate vaccines (Hib) (3), linking cap-
sular PS to proteins to provide T cell help (4) results in higher-avidity antibodies (5),
immunologic memory (6), and longer-lived protection (7). In addition, as shown in
several studies, population-wide use of Hib (8), MenA (9), and MenC (10) PS-conjugate
vaccines provided herd protection by decreasing carriage and disease among both the
vaccinated and unvaccinated. As a result, disease caused by these bacteria can be
largely controlled at the population level.

Widespread use of meningococcal PS-conjugate vaccines against MenC or MenA-
CYW has left MenB strains, for which there is no equivalent PS-conjugate vaccine, as the
cause of a majority of meningococcal disease cases in North America and Europe (11).
Strain-specific outer membrane vesicle (OMV) vaccines have been developed and used
to stem outbreaks of MenB disease, but data on the effect of OMV vaccine-elicited
antibodies on colonization are inconclusive or largely negative (12–15). Recently,
vaccines containing neisserial human complement factor H binding protein (FHbp)
have been licensed in the United States (16), and they provide much broader protection
than OMV vaccines against MenB strains, as well as strains from other meningococcal
capsular groups. The Pfizer vaccine (Trumenba, MenB-FHbp) contains two recombinant
lipid-modified FHbp antigens, one each from two sequence variant subfamilies A and
B. The GSK vaccine (Bexsero, MenB-4C) contains OMV and three recombinant protein
antigens: FHbp from subfamily B, neisserial adhesin A (NadA), and neisserial heparin
binding antigen (NHBA). Since the vaccines are relatively new and have not been used
in large populations, little is known about their effects on meningococcal carriage and
herd protection (17).

The control of meningococcal disease in large populations appears to depend
mainly on the ability of antibodies elicited by capsular PS-protein conjugate vaccines to
reduce carriage (18, 19). While several studies have described the overall effect of
meningococcal PS-conjugate vaccines on carriage, little is known about the direct
effects of the antibodies on colonizing bacteria. The purpose of this study was to
investigate, mechanistically, the effects of IgG antibodies elicited by a MenC PS-
conjugate vaccine on bacteria in a polarized airway epithelial cell model of menin-
gococcal colonization compared to antibodies elicited by plain PS, OMV, and MenB-FHbp.
In the following, we show that high-avidity IgG elicited by PS-protein conjugate
vaccines was unique in affecting characteristics of colonizing MenB and MenC strains
that limit the ability to cause disease and to disseminate between individuals.

## RESULTS

Meningococcal 16HBE14o- colonization model. To establish a model for the
initial stage of meningococcal colonization with wild-type encapsulated MenB and
MenC strains, we tested the ability of the bacteria to form colonies on Calu-3, CFBE41o-,
H441, and 16HBE14o- airway epithelial cell lines. Bacteria were added to the apical
surface of confluent cell monolayers on Transwell inserts and incubated in chemically
defined cell culture medium containing human serum albumin under immersed culture
conditions. CFBE41o- and 16HBE14o- cell lines form tight epithelial cell monolayers,
which were confirmed by electrical resistance measurements and expression of zona
occludens protein 1 (20). The bacteria in both the apical (top) and basolateral (bottom)
sides of the chamber, as well as on the epithelial cell layer, were counted after 4 h, 8
h, and 16 h of incubation. Using a ratio of approximately ~250 bacteria per 1 million
epithelial cells as a reasonable approximation of human colonization, with respect to
the number of epithelial cells being in large excess over the number of bacteria,
16HBE14o- cells gave the greatest and most consistent number of colonizing bacteria
after 4 h of incubation (data not shown), which simulates the initial stage of menin-
gococcal colonization. While invasion of the monolayer occurred as early as 4 h (MenC
strain 4243), no bacteria were detected in the lower chamber up to 8 h after adding the
bacteria.
Although secretory IgA elicited by immunization with meningococcal vaccines may have an important role in colonization, such antibodies were not available for our study. Alternatively, we used IgG, which is also present in mucosal secretions of vaccinated individuals (21). With anti-MenC PS IgG, we used amounts similar to those measured in mucosal secretions (21). The levels of IgG in the mucosa elicited by the protein-based meningococcal vaccines are not known. Therefore, we used amounts that are likely in excess of what would be expected from studies of capsular PS-protein conjugate vaccines as a best possible case test.

**Effect of vaccine-elicited IgG on meningococcal colonization of 16HBE14o-monolayers.** We purified IgG antibodies from pooled serum from children 9 months to 2 years old and adult subjects immunized with plain MenC PS vaccine (MenC PS), as well as adult subjects immunized with a MenC PS-conjugate vaccine (MenC-conjugate); then, we investigated the effect of IgG treatment on the adherence of MenC strain 4243 to 16HBE14o-cells during a 4-h incubation at 37°C. The controls included no treatment or treatment with IgG purified from serum from adults immunized with an irrelevant PS-conjugate vaccine (lrr-conjugate). Except for the no-treatment group, the amount of anti-MenC PS activity and total IgG concentration (adjusted with pre-MenC-conjugate IgG as nonimmune IgG when necessary) were the same for each treatment. As shown in Fig. 1A, the addition of post-MenC-conjugate IgG increased the number of CFU
recovered from the 16HBE14o- monolayer, while the effects of the other IgG treatments were not significantly different from those of the no-treatment group. Conversely, the CFU in the apical fluid layer decreased with MenC-conjugate IgG treatment, whereas the CFU in all the other IgG treatments were no different than in the no-treatment group (Fig. 1B). The decreased CFU measured with MenC-conjugate IgG treatment did not appear to result from aggregates formed because of anticapsular antibody cross-linking of bacteria, since we did not observe any differences in the aggregation state of bacteria with anticapsular antibodies compared to no treatment or any treatment tested by microscopy or flow cytometry.

To determine the effect of purified IgG antibodies elicited by MenB-FHbp and OMV vaccines on colonization, we used MenB strain H44/76, which is a high expressor of FHbp, and our isolate of the strain used to prepare the OMV vaccine. The subfamily B FHbp antigen used in the MenB-FHbp vaccine is 93% identical to that of FHbp ID1 produced by H44/76, and IgG antibodies elicited by MenB-FHbp bind to H44/76, as determined by flow cytometry (data not shown). There were no significant differences in the number of colonizing bacteria treated with pooled pre- or post-OMV or MenB-FHbp IgG (Fig. 1C). Since MenB PS is chemically similar to human polysialic acid and is poorly immunogenic in humans, an equivalent pool of human anti-MenB PS IgG to compare with MenC-conjugate IgG was not available. However, treatment with a mixture of two murine anti-MenB PS monoclonal antibodies (MAbs) had a similar effect on MenB strain H44/76 as post-MenC-conjugate IgG had on MenC strain 4243, where the MAb treatment increased the number of adherent CFU and decreased CFU in the apical fluid (Fig. 1C and D, respectively). We also tested MenC strain 4243 treatment with post-MenB-FHbp IgG, but there were no effects on the number of adherent or apical solution bacteria compared to no-treatment or pre-MenC-conjugate controls (data not shown).

Anticapsular antibodies inhibit shedding of capsular PS by MenC strain 4243 and MenB strain H44/76 colonizing 16HBE14o- cells. Previous studies indicated that meningococci invading epithelial cells are unencapsulated (22, 23); therefore, we investigated the effect of IgG treatments on MenC strain 4243 and MenB strain H44/76 capsular PS when grown in the presence of 16HBE14o- cells at 37°C using fluorescence microscopy. As a control, bacterial colonies grown in the absence of 16HBE14o- cells on poly-L-lysine-coated coverslips were fully encapsulated (Fig. 2A). In contrast, bacterial colonies attached to 16HBE14o- cells and treated with Irr-conjugate, adult MenC PS, or pre-MenC-conjugate IgG were less encapsulated (Fig. 2B), whereas bacteria treated with MenC-conjugate IgG retained capsular PS (Fig. 2B). Since lower temperature may decrease the production of capsular PS (24), we also looked at the MenC strain grown at 32°C in the presence of 16HBE14o- cells. As observed at the higher temperature, the bacteria were fully encapsulated when treated with post-MenC-conjugate IgG but almost completely unencapsulated in the presence of pre-MenC-conjugate IgG (Fig. 2C). The capsular polysaccharide remaining on relatively unencapsulated bacteria was concentrated in discrete areas and not colocalized with anti-porin PorA immunofluorescence, which marks the outer membrane (Fig. 2B). Also, particulate blebs of capsular polysaccharide shed from the bacteria were observed around colonies on the surface of the 16HBE14o- cells (data not shown). Taken together, the results show that the loss of capsular polysaccharide occurred by shedding rather than decreased production.

Similarly, MenB strain H44/76 colonies were fully encapsulated when grown on poly-L-lysine-coated coverslips (Fig. 3A) but less encapsulated when cultured in the presence of 16HBE14o- cells alone or treated with MenB-FHbp IgG from an individual high-responder-as-best-case test or pre- or post-OMV IgG. As was the case with post-MenC-conjugate IgG, a mixture of two anticapsular MenB PS MAbs, combined with pre-OMV IgG as a control for the presence of human IgG (25), resulted in capsule retention (Fig. 3B). In contrast to the results with anti-PorA described above, staining marked by anti-FHbp murine MAb Jar5 was colocalized with capsular polysaccharide both on the bacterial membrane of MenB strain H44/76 and in blebs shed from the
FIG 2 Fluorescence microscopy of MenC strain 4243 colonies. (A) Bacteria grown in cell culture medium in the absence of 16HBE14o- cells on coverslips. (B) Adherent bacteria colonizing 16HBE14o- cells after 4 h of incubation at 37°C and treated with the pool of purified IgG indicated on the left side of each row. (C) Adherent bacteria colonizing 16HBE14o- cells after 4 h of incubation at 32°C and treated with the pool of purified IgG indicated on the left of each panel. Blue, DAPI DNA stain; green, porin PorA P1.2; red, MenC capsular polysaccharide. Anticapsular antibodies elicited by MenC-conjugate promote the retention of capsular polysaccharide, which is otherwise shed in the presence of 16HBE14o- cells.
bacteria (see, for example, Fig. 3B, no treatment, indicated by arrows). The difference may reflect the fact that FHbp and capsular polysaccharide are associated with the outer membrane through lipid modifications, and there are potentially different mechanisms for the release of embedded versus associated outer membrane components during colonization.
Loss of capsular polysaccharide in the presence of 16HBE14o- cells does not require adhesion to the cells. Next, we asked the question of whether the loss of capsular PS by MenC strain 4243 in the presence of 16HBE14o- cells required adhesion to the cells. After a 4-h incubation with 16HBE14o- cells, nonadherent bacteria in medium above the monolayer were collected and observed by fluorescence microscopy for the presence of capsular PS. As shown in Fig. 4, the nonadherent bacteria retained capsule in the presence of post- but not pre-MenC-conjugate IgG. However, conditioned medium collected from the apical solution above the 16HBE14o- monolayer after 4 h of incubation in the absence of bacteria did not induce shedding of capsular PS when the bacteria were grown for an additional 4 h in the absence of the 16HBE14o- cells (data not shown). Also, conditioning the medium with bacteria in the absence of 16HBE14o- cells, removing the bacteria, and growing a fresh inoculum of bacteria in the conditioned medium for 4 h did not result in capsular PS shedding. Therefore, the mechanism triggering the shedding of capsular PS requires the presence of both 16HBE14o- cells and bacteria but not direct adhesion of bacteria to the epithelial cell surface.

Effect of vaccine-elicited IgG on invasion of 16HBE14o- cells. Last, we investigated whether IgG treatments had an effect on the ability of meningococci to invade 16HBE14o- cells. Bacteria invading 16HBE14o- cells after 8 h of incubation at 37°C were detected by resistance to gentamicin treatment. Antibody treatments included no IgG (both strains), pre- or post-IgG from MenC-conjugate (MenC strain 4243), MenB-FHbp high-responding individual, and OMV (MenB strain H44/76) sera. Additional controls for strain H44/76 included mixtures of mouse anti-PorA P1.7 and anti-FHbp or anti-MenB PS MAb. Intracellular bacteria were recovered for both strains (4243, Fig. 5A; H44/76, Fig. 5B and C). Only post-MenC-conjugate IgG and mouse anti-MenB PS MAb treatments significantly inhibited the invasion of MenC (Fig. 5A) and MenB (Fig. 5B).

DISCUSSION

The introduction of PS-protein conjugate vaccines has had a profound effect on controlling disease caused by encapsulated bacterial pathogens in large populations. Among the most important benefits of these vaccines has been their ability to reduce carriage and disease through herd protection in unvaccinated individuals. While herd protection was demonstrated in many epidemiological studies (3, 8–10), the mechanisms by which PS-conjugate vaccine-elicited antibodies exert this effect remained unknown. The question of mechanism has become particularly important with the introduction of protein-based vaccines to protect against disease caused by MenB, whose capsular polysaccharide is similar to host polysialic acid, thus precluding the development of MenB PS-conjugate vaccines (26, 27). To address the question of mechanism, we characterized the effect of MenC-conjugate-elicited IgG compared to that with IgG elicited by other vaccines known to not affect colonization or provide
herd protection or, in the case of the newly licensed “MenB vaccines” (16), to have unknown effects on colonization.

In the 16HBE14o- model, we observed that colonizing MenB and MenC strains shed capsule by blebbing capsular PS. Membrane blebbing has long been recognized as a characteristic of meningococcal strains that cause invasive disease (28). Also, studies of cellular invasion by meningococci in human tissue explants showed that bacteria invading epithelial cells lacked capsular PS, while neighboring noninvading bacteria

**FIG 5** Effect of IgG on meningococcal invasion of 16HBE14o- cells by MenC strain 4243 (A) and MenB strain H44/76 (B and C). Invading bacteria were identified by resistance to gentamicin treatment. CFU per milliliter is the number of bacteria in the 200 μl of trypsin solution. The effects of no treatment and pre- and post-MenC-conjugate IgG were compared for MenC strain 4243 (A). The effects of no treatment or treatment with murine MAbs (anti-PorA P1.7, anti-FHbp Jar5, antcapsular SEAM 2, and SEAM 12), pre- and post-OMV IgG (B), and pre- and post-MenB-FHbp IgG from a single high responder (C) were compared for MenB strain H44/76. Each bar represents the mean ± SEM from 4 biological replicates. ***, P < 0.001 for postimmune compared to preimmune IgG or no treatment; **, P < 0.01 for anti-MenB PS MAbs compared to anti-PorA/FHbp MAbs. Only antcapsular IgG (post-MenC-conjugate and antcapsular MenB PS MAbs) elicited by PS-conjugate vaccines inhibited bacterial invasion of the 16HBE14o- cells.
bound to cilia retained capsule (22, 23). Several mechanisms have been described for the regulation of capsular PS production during epithelial cell invasion, including phase variation (29, 30), misR-misS two-component regulation of capsular operon gene expression (31), and temperature-dependent formation of mRNA hairpin structures that reduce expression of the polysialyltransferase at low temperature (24). However, in the 16HBE14o- model, the amount of capsular PS was not determined by regulating production but instead by shedding with MenC-conjugate IgG or anticapsular MenB PS MAb5 inhibiting shedding. Shedding of the capsule did not depend on adhesion to the epithelial cell surface but required both 16HBE14o- cells and bacteria, suggesting the existence of an unknown soluble signaling molecule and bacterial system to regulate capsule shedding. Also, there may be differences in the mechanism of blebbing outer membrane containing integral membrane protein PorA versus lipid-modified capsular PS and FHbp, since blebs of lipid-modified capsular PS and FHbp were always colocalized by fluorescence microscopy, while blebs of PorA and capsular PS were not.

MenC-conjugate IgG and murine anti-MenB PS MAbs were unique among the vaccine-elicited IgG studied in the ability to prevent loss of capsule, increase the number of bacteria adherent to 16HBE14o- cells, decrease the number of bacteria in the fluid layer on the apical side of the cell monolayer, and inhibit invasion of the cell monolayer. Also, although we did not quantify the effect, treatment with PS-conjugate vaccine-elicited anticapsular antibodies resulted in adherent bacterial colonies that were qualitatively more condensed than no-IgG or other IgG treatments, suggesting that the high-avidity anticapsular antibodies inhibit microcolony dispersal as well (32). These effects may contribute to reducing carriage by making adherent encapsulated bacteria more efficiently killed by complement or cellular-dependent mechanisms or washed out by the flow of mucus in the airway. Also, reducing the number of bacteria in the fluid layer above the cell monolayer may have a similar effect of reducing bacteria in mucosal secretions that can be spread by aerosol droplets. It is unclear how anticapsular IgG blocks the loss of capsule, but we suggest that cross-linking of polysaccharide chains by high-avidity polyclonal IgG likely contributes to this effect. The ability of PS-conjugate vaccines to elicit high-avidity antibodies distinguishes these vaccines from plain PS, which elicits antibodies of generally lower avidity, particularly in infants (33).

In summary, this study provides new insights on how interactions of meningococcal strains with airway epithelial cells affect capsule shedding as a prerequisite to cellular invasion and inhibition of shedding by PS-conjugate vaccine-elicited IgG, as well as how these effects limit the potential for pathogenic meningococcal strains to spread among individuals. Given the unique effect of PS-conjugate vaccine-elicited IgG on capsule retention and characteristics of colonizing bacteria, it is clear that antibodies elicited by the existing recombinant FHbp protein- or OMV-based vaccines would affect carriage by a different mechanism, since they had no effect on capsule retention. Likely, protein vaccines will need to contain other antigens directly involved in colonization, such as conserved adhesins, in order to affect carriage and thus provide some measure of herd protection.

MATERIALS AND METHODS

Immunoglobulin G purification. Donated human blood used in this study was obtained from donors under a protocol approved by the UCSF Benioff Children's Hospital Oakland institutional review board, with written informed consent obtained from all adult donor participants or from the legal guardians of the children who participated.

Pools were made from sera obtained from children 9 months to 2 years old (n = 7) and adults (n = 4) immunized with the ACYW quadrivalent PS (MenC PS) vaccine (34), sera from adults immunized with irrelevant pneumococcal 7-valent PS-conjugate vaccine (Prevnar) (n = 7) (35) (Irr-conjugate), and pre- and postvaccination sera from adults immunized with a MenC PS-CRM197 conjugate (MenC-conjugate) vaccine (n = 6) (36), outer membrane vesicle (OMV) vaccine (n = 5), and MenB-FHbp vaccine (n = 4). Pre- and postvaccination sera were also used from an individual who had a high response to the MenB-FHbp vaccine as a best-case test in some experiments. Immunoglobulin G (IgG) from the pooled sera was purified using separate 5-ml HiTrap protein G HP columns (GE Healthcare Bio-Sciences, Marlborough, MA, USA) on an Akta fast-performance liquid chromatograph (FPLC; GE Healthcare Bio-Sciences). The IgG was eluted in 0.1 M histidine (pH 2.7) containing 0.02% (wt/vol) Tween 20 and immediately neutralized with...
comparisons were made as described above. For the pools of subjects immunized with MenC PS, MenC-conjugate, and Irr-conjugate vaccines, an enzyme-linked immunosorbent assay (ELISA) was done to determine the amounts of specific anti-MenC PS binding activity, as described previously (36). Binding activity was expressed in micrograms of MenC PS-specific IgG based on a comparison to human reference serum CDC1992 obtained from the National Institute for Biological Standards and Control, Hertfordshire, United Kingdom.

Human bronchial epithelial cells. The human epithelial cell line 16HBE14o- was originally obtained from a 1-year-old heart-lung transplant patient and immortalized with simian virus 40 (SV40) large T antigen by using the replication defective pSVori- plasmid (37). 16HBE14o- cells were kindly provided by D. C. Gruenert (University of California, San Francisco, CA) at passage 2.45 and cultured as previously described for transplanted chloride ion transport measurements (38). For this study, cells were seeded on clear Transwell inserts (polyester membrane, 12-mm diameter, 0.4-μm or 3.0-μm pore size; Corning, Corning, NY, USA) that were previously coated with a mixture of fibronectin-vitrogen-bovine serum albumin in LHC basal medium (Thermo Fisher Scientific, Waltham, MA) (39, 40). Cells were plated at a density of 1 × 10⁴ cells/cm² and grown to confluence in Eagle’s minimal essential medium (Invitrogen, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS; HyClone Characterized; GE Healthcare Life Sciences), 100 U/ml penicillin, 100 μg/ml streptomycin (UCSF Cell Culture Facility, San Francisco, CA, USA), and 2 mM glutamine (UCSF Cell Culture Facility). 16HBE14o- cell monolayers were grown under immune culture conditions, with an apical volume of 500 μl and a basolateral volume of 1 ml to facilitate the formation of higher transepithelial electrical resistance values, as pointed out by Ehrhardt et al. (20). Cell monolayers were incubated in antibiotic-free medium (minimal essential medium (MEM), glutamine, and FBS) overnight, followed by 1:10 and FBS) ovalbumin periods with MEM, 4 mM D,L-lactate (Sigma-Aldrich, St. Louis, MO, USA), and 1 mg/ml human serum albumin (Sigma-Aldrich) before the start of each experiment.

Colonization assay. N. meningitidis strains were grown overnight on chocolate agar plates (Remel, Lenexa, KS) at 37°C with 5% CO₂. Liquid cultures were then grown to mid-log phase in Frantz medium supplemented with 4 mM D,L-lactate. The bacteria were diluted 1:1,000,000 in MEM containing 1 mg/ml human serum albumin and 4 mM D,L-lactate. In each assay, the IgG treatments were adjusted to 0.05 μg of anti-MenC PS and 400 μg of total IgG with pre-MenC-conjugate IgG. The amount of MenC-conjugate IgG chosen for testing was based on preliminary experiments where the antibody was serially diluted and the effects on colonization (i.e., the number of bacteria in the medium described above and adhering to described for transplanted chloride ion transport) were determined. We then used an amount that consistently produced the results shown in Fig. 1A and 2B of MenC-conjugate for each of the treatments. The IgG pools of subjects immunized with the OMV and MenB-FHbp vaccines were used at a final concentration of 2.5%. The amounts of MenB antisera capsular MAb's, OMV, and MenB-FHbp IgG were arbitrary, since there was no effect on capsule retention, but the amounts were approximately 10-fold higher than serum bactericidal titers determined against the same strains with human complement. The amount of IgG based on a comparison to human reference serum CDC1992 obtained from the National Institute for Biological Standards and Control, Hertfordshire, United Kingdom.

Conditioned medium. Transwell inserts with 16HBE14o- cell monolayers were incubated for 4 h in medium (MEM, 1 mg/ml human serum albumin, and D,L-lactate) for conditioning. The liquid above the cell monolayer was aspirated, the wells were washed twice with 500 μl of phosphate-buffered saline (PBS) containing 0.01% Tween 20, and 200 μl of 0.05% trypsin solution (UCSF Cell Culture Facility) was added. The plates were incubated on a shaker for 15 min at 37°C. The samples were then spotted on chocolate agar plates and tipped up to spread the solution. Viable CFU were counted after overnight incubation. Statistical comparison by two-tailed Mann-Whitney test was performed using GraphPad Prism version 7.0a for Mac OSX (GraphPad Software, Inc., San Diego, CA, USA).

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Invasion assay. After 8 h of incubation with or without IgG treatments, at 37°C with 5% CO₂, the Transwell inserts with 16HBE14o- cell monolayers were washed twice with 500 μl of PBS with 0.01% Tween 20, followed by the addition of 200 μl of 200 μg/ml gentamicin (Thermo Fisher Scientific) in medium (MEM, 1 mg/ml human serum albumin, and D,L-lactate). After 1 h of incubation, the gentamicin was aspirated, the Transwell inserts were washed three times with 500 μl of Dulbecco’s PBS (DPBS), and 200 μl of 0.05% trypsin was added. The plates were incubated on a shaker for 15 min at 37°C. The trypsin solution (10 μl) was spotted on chocolate agar plates and counted as described above. Statistical comparisons were made as described above.

Microscopy. Coverslips were coated with polylysine (Sigma-Aldrich) at a concentration of 1 mg/ml and dried at room temperature overnight before use. They were washed three times with sterile DPBS before adding bacteria. The liquid above the cell monolayer in Transwell inserts was aspirated after the 4-h incubation, the wells were washed twice with 500 μl of PBS and 0.01% Tween 20, and 500 μl of 4% paraformaldehyde was added. After a 20-min incubation, the liquid was aspirated, and 500 μl of blocking
buffer (2% goat serum in DPBS and 0.02% Tween 20) was added for an overnight incubation at 4°C. Nonadherent bacteria from the colonization assay and bacteria from the conditioned medium assay were dried on plain coverslips after centrifugation and subsequent suspension using 4% paraformaldehyde. The coverslips were then blocked overnight as described above. Both the coverslips and the Transwell inserts were marked for PorA (P1.2 for 4243, P1.16 for H44/76), capsular PS JW-C1 for 4243, SEAM 12 [41] for H44/76, factor H (sheep polyclonal antibody to factor H; Abcam, Cambridge, MA, USA), or factor H binding protein (Jar5), with detection using goat anti-mouse IgG Alexa Fluor 488 and goat anti-mouse IgG Alexa Fluor 594 subclass-specific secondary antibodies and 4’,6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific) to stain the DNA. Images were recorded on a Zeiss LSM-710 confocal microscope (Zeiss, Oberkochen, Germany).

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REFERENCES

1. Carbonnelle E, Hill DJ, Morand P, Griffiths NJ, Bourdoulous S, Murillo I, Nassif X, Virji M. 2009. Meningococcal interactions with the host. Vaccine 27(Suppl 2):S87–S89. https://doi.org/10.1016/j.vaccine.2009.04.069.
2. Maiden MC. 2013. The impact of protein-conjugate polysaccharide vaccines: an endgame for meningitis? Philos Trans R Soc Lond B Biol Sci 368:20120147. https://doi.org/10.1098/rstb.2012.0147.
3. Murphy TV, Pastor P, Medley F, Osterholm MT, Granoff DM. 1993. Decreased Haemophilus colonization in children vaccinated with Haemophilus influenzae type b conjugate vaccine. J Pediatr 122:517–523. https://doi.org/10.1016/S0022-3476(05)83529-2.
4. Schneerson JR, Barrera O, Sutton A, Robbins JB. 1980. Preparation, characterization, and immunogenicity of Haemophilus influenzae type b polysaccharide-protein conjugates. J Exp Med 152:361–376. https://doi.org/10.1084/jem.152.2.361.
5. Schlesinger Y, Granoff DM. 1992. Avidity and bactericidal activity of antibody elicited by different Haemophilus influenzae type b conjugate vaccines. The Vaccine Study Group. JAMA 267:1499–1499.
6. Granoff DM, Holmes SJ, Osterholm MT, McHugh JE, Lucas AH, Anderson EL, Belshe RB, Jacobs JL, Medley F, Murphy TV. 1993. Induction of immunologic memory in infants primed with Haemophilus influenzae type b conjugate vaccines. J Infect Dis 168:663–671. https://doi.org/10.1093/infdis/168.3.663.
7. Blanchard-Rohner G, Pollard AJ. 2011. Long-term protection after immunization with protein-polysaccharide conjugate vaccines in infancy. Expert Rev Vaccines 10:673–684. https://doi.org/10.1586/erv.11.14.
8. Murphy TV, White KE, Pastor P, Gabriel L, Medley F, Granoff DM, Osterholm MT. 1993. Declining incidence of Haemophilus influenzae type b disease since introduction of vaccination. JAMA 269:246–248. https://doi.org/10.1001/jama.1993.035002080036.
9. Kristiansen PA, Diomande F, Ba AK, Sanou I, Ouedraogo IS, Ouedraogo K, Sangare L, Kandolo D, Ake F, Saga IM, Clark TA, Misegades L, Martin SW, Thomas JD, Tiendrebeogo SR, Hassan-King M, Djingarey MH, Monsenner NE, Prezioso MP, Laforce FM, Caugant DA. 2013. Impact of the serogroup A meningococcal conjugate vaccine, MenAfriVac, on carriage and herd immunity. Clin Infect Dis 56:354–363. https://doi.org/10.1093/cid/cis892.
10. Ramsay ME, Andrews NJ, Trotter CL, Kaczmarski EB, Miller E. 2003. herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis. BMJ 326:365–366. https://doi.org/10.1136/bmj.326.7385.365.
11. Sridhar S, Greenwood B, Head C, Plotkin SA, Safadi MA, Saha S, Taha MK, Tomori O, Gessner BD. 2015. Global incidence of serogroup B invasive meningococcal disease: a systematic review. Lancet Infect Dis 15:1334–1346. https://doi.org/10.1016/S1473-3099(15)00217-0.
12. Bjune G, Gronnesby JK, Holby EA, Coss O, Nokleby H. 1991. Results of an efficacy trial with an outer membrane vesicle vaccine against systemic serogroup B meningococcal disease in Norway. NIPH Ann 14:125–130, discussion 130–132.
13. Wedege E, Kuipers B, Bostad K, van Dijken H, Froholm LO, Vermont C, Caugant DA, van den Dobblesteen G. 2003. Antibody specificities and effect of meningococcal carriage in Icelandic teenagers receiving the Norwegian serogroup B outer membrane vesicle vaccine. Infect Immun 71:3775–3781. https://doi.org/10.1128/IAI.71.7.3775-3781.2003.
14. Davenport V, Groves E, Horton RE, Hobbs CG, Guthrie T, Findlow J, Borrow R, Naess LM, Oster P, Heyderman RS, Williams NA. 2008. Mucosal immunity in healthy adults after parenteral vaccination with outer-membrane vesicles from Neisseria meningitidis serogroup B. J Infect Dis 198:731–740. https://doi.org/10.1086/jvaccine.2013.06.080.
15. Delbos V, Lemee L, Benichou J, Berthelot G, Dегhmаnе AE, Lеrоу JP, Houivet E, Hong E, Taha MK, Caron F, B14 STOP Study Group. 2013. Impact of MenBvac, an outer membrane vesicle (OMV) vaccine, on the meningococcal carriage. Vaccine 31:4416–4420. https://doi.org/10.1016/j.vaccine.2013.06.080.
16. Folaranmi T, Rubin L, Martin SW, Patel M, MacNeil JR, Centers for Disease Control (CDC). 2015. Use of serogroup B meningococcal vaccines in persons aged ≥10 years at increased risk for serogroup B meningococcal disease: recommendations of the Advisory Committee on Immunization Practices, 2015. MMWR Morb Mortal Wkly Rep 64:608–612.
17. Read RC, Dull P, Bai X, Nolan K, Findlow J, Bazaz R, Kleinschmidt A, McCrory M, Wang H, Toneatto D, Borrow R. 2017. A phase III observer-blind randomized, controlled study to evaluate the immune response and the correlation with nasopharyngeal carriage after immunization of university students with a quadrivalent meningococcal ACWY glycoconjugate or serogroup B meningococcal vaccine. Vaccine 35:427–434. https://doi.org/10.1016/j.vaccine.2016.11.071.
18. Barbour ML, Mayon-White RT, Coles C, Crook DW, Moxon ER. 1995. The impact of conjugate vaccine on carriage of Haemophilus influenzae type b. J Infect Dis 171:93–98. https://doi.org/10.1093/infdis/171.1.93.
19. Maiden MC, Ibarz-Pavon AB, Urwin R, Gray SJ, Andrews NJ, Clarke SC, Walker AM, Evans MR, Kroll JS, Neal KR, Ala’aldene DA, Crook DW, Kann C, Harrison S, Cunningham R, Baxter D, Kaczmarski E, MacLennan J, Cameron JC, Stuart JM. 2008. Impact of meningococcal serogroup C...
24. Loh E, Kugelberg E, Tracy A, Zhang Q, Gollan B, Ewles H, Chalmers R, Stephens DS. 1989. Gonococcal and meningococcal pathogenesis as
23. defined by human cell, cell culture, and organ culture assays. Clin Microbiol Rev 2(Suppl):S104–S111.
22. Loh E, Kugelberg E, Tracy A, Zhang Q, Gollan B, Ewles H, Chalmers R, Stephens DS. 1989. Invasion of primary nasopharyngeal epithelial cells by Neisseria meningitidis is controlled by phase variation of multiple surface antigens. Infect Immun 64:2998–3006.
21.

20. Ethhardt C, Kneuer C, Fiegel J, Hanes J, Schaefler UF, Kim KJ, Lehr CM. 2002. Influence of apical fluid volume on the development of functional intercellular junctions in the human epithelial cell line 16HBE14o–: implications for the use of this cell line as an in vitro model for bronchial drug absorption studies. Cell Tissue Res 308:391–400. https://doi.org/10.1007/s00441-002-0548-5.
19.

18. Nakano TA, Steirer LM, Moe GR. 2011. The expression profile of de
17. 2017 Volume 24 Issue 10 e00188-17 cvi.asm.org
16. 2014. Meningococcal group C conjugate vaccination. Clin Vaccine Immunol 13:605–610. https://doi.org/10.1128/CVI.00123-06.
15. Vu DM, Welsch JA, Zuno-Mitchell P, Dela Cruz JV, Granoff DM. 2006. Antibody persistence 3 years after immunization of adolescents with quadrivalent meningococcal conjugate vaccine. J Infect Dis 193:821–828. https://doi.org/10.1086/500512.
14. 1988. Characterization of human tracheal epithelial cells transformed by an origin-defective simian virus 40. Proc Natl Acad Sci USA 85:5951–5955. https://doi.org/10.1073/pnas.85.16.5951.
13. 1996. Characterization of human tracheal epithelial cells transformed by an origin-defective simian virus 40. Proc Natl Acad Sci USA 85:5951–5955. https://doi.org/10.1073/pnas.85.16.5951.
12. 1988. Characterization of human tracheal epithelial cells transformed by an origin-defective simian virus 40. Proc Natl Acad Sci USA 85:5951–5955. https://doi.org/10.1073/pnas.85.16.5951.
11. Vu DM, Welsch JA, Zuno-Mitchell P, Dela Cruz JV, Granoff DM. 2006. Antibody persistence 3 years after immunization of adolescents with quadrivalent meningococcal conjugate vaccine. J Infect Dis 193:821–828. https://doi.org/10.1086/500512.
10. 1996. Characterization of immortal cystic fibrosis tracheobronchial gland epithelial cells. Proc Natl Acad Sci USA 89:5171–5175. https://doi.org/10.1073/pnas.89.11.5171.
9. 2010. Sensitivity of chloride efflux vs. transepithelial measurements in mixed CF and normal airway epithelial cell populations. Cell Physiol Biochem 26:983–990. https://doi.org/10.1159/000324011.
8. Hammerschmidt S, Hilsie R, van Putten JP, Gerardy-Schahn R, Unkmeir A, Frosch M. 1996. Modulation of cell surface sialic acid expression in Neisseria meningitidis via a transposable genetic element. EMBO J 15:192–198.
7. Hammerschmidt S, Muller A, Sillmann H, Muhlenhoff M, Borrow R, Fox A, van Putten J, Zollinger WD, Gerardy-Schahn R, Frosch M. 1996. Capsule phase variation in Neisseria meningitidis serogroup B by slipped-strand mispairing in the polysialyltransferase gene (siaD): correlation with bacterial invasion and the outbreak of meningococcal disease. Mol Microbiol 20:1211–1220. https://doi.org/10.1111/j.1365-2958.1996.tb02641.x.
6. 1996. Modulation of cell surface sialic acid expression in Neisseria meningitidis via a transposable genetic element. EMBO J 15:192–198.
5. Müller MG, Moe NE, Richards PQ, Moe GR. 2015. Resistance of Neisseria meningitidis to human serum depends on T and B cell stimulating protein B. Infect Immun 83:1257–1264. https://doi.org/10.1128/IAI.03134-14.
4. Frosch M. 1996. Modulation of cell surface sialic acid expression in Neisseria meningitidis via a transposable genetic element. EMBO J 15:192–198.
3. 2004. Protective activity of group C capsular antibodies elicited in two-year-olds by an investigational quadrivalent Neisseria meningitidis-diphtheria toxoid conjugate vaccine. Pediatr Infect Dis J 23:490–497. https://doi.org/10.1097/00002988.2004.006.
2. Granoff DM, Harris SL. 2004. Protective activity of group C capsular antibodies elicited in two-year-olds by an investigational quadrivalent Neisseria meningitidis-diphtheria toxoid conjugate vaccine. Pediatr Infect Dis J 23:490–497. https://doi.org/10.1097/00002988.2004.006.