Susceptibility of Meningococcal Strains Responsible for Two Serogroup B Outbreaks on U.S. University Campuses to Serum Bactericidal Activity Elicited by the MenB-4C Vaccine

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Abstract

In 2013-14, two U.S. universities had meningococcal serogroup B outbreaks (total of 14 cases) caused by strains from different clonal complexes. To control the outbreaks, students were immunized with a serogroup B meningococcal vaccine (Novartis) that was not yet licensed in the U.S. The vaccine (referred to as “MenB-4C”) contains four components capable of eliciting bactericidal activity. Both outbreak strains had high expression of two of the vaccine antigens (sub-family B FHbp and NHba); the University B outbreak strain also had moderate expression of a third antigen, NadA. We investigated bactericidal activity of sera from mice immunized with FHbp, NHba or NadA, and sera from MenB-4C-immunized infant macaques and an adult human. The post-immunization bactericidal activity of the macaque or human sera was 8 to 21-fold higher against isolates from University B with FHbp ID 1 that exactly matched the vaccine FHbp sequence variant than isolates from University A with FHbp ID 276 (96% identity to the vaccine antigen). Based on bactericidal activity of mouse antisera to FHbp, NadA or NHba, and macaque or human post-immunization serum that had been depleted of anti-FHbp antibody, bactericidal activity against both outbreak strains largely or entirely resulted from antibodies to FHbp. Thus, despite high strain expression of FHbp from a sub-family that matched the vaccine antigen there can be large differences in anti-FHbp bactericidal activity induced by MenB-4C vaccination. Further, strains with moderate to high NadA and/or NHba expression can be resistant to anti-NadA or anti-NHba bactericidal activity elicited by MenB-4C vaccination.
Introduction

Meningococcal serogroup B outbreaks involving a total of 14 cases occurred on two university campuses in the United States in 2013-2014 (1). The outbreak in University A, which is located in New Jersey, started in March 2013 with a total of 9 cases documented in the campus population or in close contacts of the students (2). The outbreak in University B, which is located in California, started in November 2013 with a cluster of four cases (1). These cases were later connected to a fifth case in a student enrolled at University B, which had occurred seven months earlier. The strains from the two outbreaks were from different clonal complexes and, therefore, were not epidemiologically related.

In response to these campus outbreaks, the U.S. Food and Drug Agency approved immunization of the students with a serogroup B meningococcal vaccine (Bexsero, Novartis Vaccines and Diagnostics; now GSK) (2), which at the time was licensed in Europe, Canada and Australia. The vaccine contains four primary components, each capable of eliciting complement-mediated serum bactericidal activity (3, 4), and is referred to herein as “MenB-4C”. The four antigens are Factor H binding protein (FHbp), Neisserial Heparin binding antigen (NHba), Neisserial adhesin A (NadA), and a porin protein (PorA) with variable region (VR) sequence type P1.7-2,4 contained in outer membrane vesicles (OMV) (3, 4). Since most of the anti-PorA bactericidal activity is directed to VR2, a “match” between the vaccine PorA and strain PorA is usually described as sharing the P1.4 VR2 antigen (5).

The purpose of the present study was to investigate expression of MenB-4C vaccine antigens in representative isolates from each of the outbreaks, and
susceptibility of the isolates to serum bactericidal activity induced by MenB-4C vaccination. We also assessed the contribution of anti-FHbp antibodies in eliciting serum bactericidal activity.

Material and Methods

Meningococcal outbreak strains. We received 15 case isolates from the Meningitis Laboratory, Meningitis and Vaccine Preventable Diseases Branch, Centers for Disease Control and Prevention (CDC), Atlanta, GA. Of these, 10 isolates were from the University A outbreak and 5 were from the University B outbreak. CDC provided data on date of isolation, source (blood or CSF), multilocus sequence type (MLST), PorB amino acid sequence type, and amino acid sequence variants of the FHbp, NadA, NHba, and PorA vaccine antigens. The isolates from University A were sequence type- (ST-) 409, which is uncommon in the U.S. but part of the more common ST-41/44 clonal complex. The isolates from University B were all ST-32, which is a common ST/clonal complex among invasive serogroup B case isolates from the west coast of the United States (6).

Control strains. The strains used as controls are summarized in Table 1 and have been previously described. Strain NZ98/254 is a relatively low expresser of FHbp ID 14 (sub-family B) (7), and was used in flow cytometric studies as a control to measure FHbp expression. The remaining control strains were each mismatched for three of the four antigens in MenB-4C known to elicit serum bactericidal activity (8-10). Thus, serum bactericidal activity elicited by the MenB-4C vaccine against strain H44/76 is specific for antibodies to FHbp; strain 5/99 for antibodies to NadA; and strain SK016 for antibodies to PorA P1.4. In past studies, finding a control strain specific for anti-
NHba bactericidal activity has been problematic (9, 11, 12). For our study we used strain M4407, which is mismatched for all of the vaccine antigens except NHba (i.e. lacks a NadA gene, has a heterologous PorA to that in the OMV in MenB-4C, and expresses sub-family A FHbp (Table 1). Strain M4407 is a high expresser of NHba (100% amino acid identity to vaccine (10)). The strain is susceptible to mouse anti-NHba serum bactericidal activity but resistant to bactericidal activity by mouse antisera to sub-family B FHbp ID 1 or NadA (See Results).

Antigen surface expression. Expression of FHbp, NHba and NadA on the surface of live meningococci was measured by flow cytometry, which was performed as previously described (13). FHbp was detected with an anti-FHbp mAb, JAR 41, which recognizes all FHbp amino acid sequence variants tested from sub-family A or B (14). Additional antibodies included mouse mAbs to the capsule (SEAM 12 (15)) or PorA P1.4, and polyclonal mouse antisera specific for the NHba and NadA antigens in MenB-4C vaccine (3) (See below).

Mouse sera. Stored sera were available from female CD-1 mice (Charles River) immunized with a recombinant sub-family B FHbp ID 1 vaccine, NadA, or the NHba-GNA1030 fusion protein used in the MenB-4C vaccine (3) using protocols described previously (10). Serum pools were made from equal volumes of sera from 3 to 5 individual mice. The mice were housed in a facility certified by AAALAC under a protocol approved by the Institutional Animal Care and Use Committee of UCSF Benioff Children’s Hospital Oakland Research Institute.

Rhesus macaque sera. We tested sera from a previous study done in infant rhesus macaques immunized beginning at 3 to 4 months of age with two doses of the
MenB-4C vaccine (16). We strictly adhered to the "Guide for the Care and Use of Laboratory Animals" (17), and the study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis. For the present study we selected post-immunization sera from five of the six vaccinated animals whose pre-immunization serum FH bound to FHbp ID 1 with similar affinity as that of human FH; the sixth animal was excluded because of an insufficient volume of serum. As negative controls, we included sera from three unvaccinated animals.

**Human serum.** As previously described (18), we obtained serum samples from a healthy adult two weeks before and six weeks after receiving a third dose of the MenB-4C vaccine. The subject had been immunized approximately 5 years earlier with two doses of MenB-4C as part of a clinical trial in Europe. Informed written consent was obtained from the subject and the studies were approved by the UCSF Benioff Children’s Hospital Oakland Institutional Review Board.

**Complement mediated serum bactericidal activity.** We measured serum bactericidal activity as described previously (19). The assay uses bacteria grown to mid-log phase in broth, and exogenous human complement consisting of serum depleted of IgG using a protein G column (19). The percentage survival of CFU/ml was calculated as a ratio of CFU/ml after 60 min incubation of dilutions of the test sera and negative control sera from the respective species.

**Depletion of serum anti-FHbp antibodies.** A post-immunization serum pool from the five rhesus macaques immunized with the MenB-4C vaccine and the post-immunization serum from the immunized human were depleted of anti-FHbp antibodies using a R41S FHbp ID1 mutant recombinant protein coupled to Sepharose as described
previously (18). As a negative control the respective sera were incubated with Sepharose beads that had not been coupled to a protein (referred to as “mock-adsorption.” The efficiency of depletion of serum anti-FHbp antibodies, and specificity of the depletion, were measured by ELISA using microtiter plates (Immulon 2B; Thermo Scientific) coated with individual recombinant wildtype FHbp ID 1, NadA or NHba, or outer membrane vesicles (OMV) prepared from a mutant of *N. meningitidis* strain NZ98/254 (4) in which the gene encoding FHbp had been inactivated.

Results

**Characteristics of outbreak isolates.** The two outbreaks were caused by epidemiologically unrelated strains that were derived from either clonal complex ST-41/44 (University A) or ST-32 (University B). The isolates from the University A outbreak had identical respective PorA, PorB, FHbp, and NHba variants as inferred from DNA sequencing, and all of the isolates lacked the gene encoding NadA. Thus, these isolates appeared to be derived from a single clone. The isolates from University A had genes encoding two of the four MenB-4C antigens (*Table 2*): sub-family B FHbp (ID 276, 96% identical to FHbp ID 1 in the MenB-4C vaccine) and NHba variant 2 [100% identical to NHba in MenB-4C (20, 21)]. The case isolates from the University B outbreak had genes encoding identical respective PorA, FHbp, NHba and NadA variants but expressed one of two related PorB sequences (PorB3-24 or PorB3-461, with 97.6% amino acid identity). The slightly different PorB sequences indicated that the University B isolates were derived from two closely-related but not identical strains. The University B isolates were matched for three of the four MenB-4C antigens: FHbp ID 1
We selected two representative blood isolates from each outbreak for further characterization of vaccine antigen expression and susceptibility to human-complement mediated serum bactericidal activity (Table 2). Selection of these isolates was based on including an early and late blood isolate from cases in each outbreak and, for University B, inclusion of one isolate each with different PorB sequence types.

Strain antigen expression. We used flow cytometry to measure surface expression of three of the MenB-4C vaccine antigens, FHbp, NadA and NHba. We did not test the fourth antigen, PorA P1.4 present in the OMV component of MenB-4C, since neither outbreak strain had the gene sequence encoding PorA 1.4.

Representative antigen expression data for one isolate from each of the outbreaks are shown in Fig. 1. Similar respective data were obtained with the second isolate tested from each outbreak (data not shown).

Both outbreak isolates showed similar binding by the flow assay using a control murine mAb to the serogroup B capsule (Fig. 1, Panel A). Both outbreak strains had higher FHbp expression than the control NZ98/254 strain (Panel B, dotted line), which is known to have relative low FHbp expression (22). The isolate from the University B outbreak had slightly more FHbp expression than the isolate from the University A outbreak (Panel B, compare solid and dashed lines, respectively).

The isolates from both university outbreaks also had high expression of NHba (Fig. 1, Panel C). The University A isolate (dashed line) had similar NHba expression as
The University B isolates expressed NadA (Fig. 1, Panel D, solid line). The amount was less than the control 5/99 strain (dotted line), which was selected as a positive control based on high NadA expression and susceptibility to anti-NadA serum bactericidal activity (9, 11) (See also Results). As expected, there was no detectable NadA expression by the isolate from the University A outbreak (dashed line), which lacked a NadA gene (Table 2).

**Susceptibility to bactericidal activity, mouse antisera.** We tested bactericidal activity of specific antisera from mice immunized with recombinant FHbp, NHba or NadA (See Methods). For each antigen, the amino acid sequence of the recombinant protein used to immunize the mice matched the respective antigenic variants used in the MenB-4C vaccine. Similar respective results were obtained with two isolates tested from each outbreak. We therefore show the data for only one isolate from each outbreak. Despite high NHba expression for both isolates, and moderately high NadA expression by the isolate from University B, the two isolates were only susceptible to human complement-mediated bactericidal activity elicited by antibodies to FHbp (Fig. 2, open bars, University A; dark gray bars, University B). The mouse anti-FHbp bactericidal titer against the isolate from University B was ~44-fold higher than the titer against the isolate from University A (see also Fig. 3, Panel B). As positive controls, the respective mouse antisera were each bactericidal against control strains (Table 1 and Fig. 2) matched for the corresponding antigen and mismatched for the other three
antigens in the MenB-4C vaccine: strain SK016 (matched for PorA P1.4); H44/76
(matched for FHbp); 5/99 (matched for NadA); and M4407 (matched for NHba).

*Rhesus macaque sera.* We measured bactericidal activity of sera from five infant
macaques immunized with two doses of the MenB-4C vaccine study. Data are
expressed as the mean percent survival of each of the test isolates when incubated with
human complement and different dilutions of post-immunization macaque sera. The
three unvaccinated negative control macaques had negative titers against the isolate
from University A (titer <1:4, lowest dilution tested), and a titer of ~1:8 against the
isolate from University B (Fig. 3, Panel C). After 2 doses of MenB-4C, the serum titer
was ~1:33 against the isolate from University A and ~1:226 against the isolate from
University B (Fig. 3, Panel D), which is an 8-fold difference.

*Human serum.* We also observed higher bactericidal titers against the isolates
from University B outbreak than the University A outbreak in serum from an adult
human immunized with a third dose of the MenB-4C vaccine (Fig. 3, Panels E and F).
This individual had received two previous doses of MenB-4C five years earlier. The
“pre” serum for this individual was obtained 2 weeks before dose 3, and had a
bactericidal titer of ~1:10 against both isolates (Panel E). The serum titers after
vaccination increased to ~1:40 against the isolate from University A, and ~1:840 against
the isolate from University B (~21-fold difference, comparing the two isolates). Similar
respective titers were obtained against second isolates from each outbreak (data not
shown).

*Effect of depletion of serum anti-FHbp antibody on bactericidal activity.* To
determine the contribution of anti-FHbp antibodies to bactericidal activity elicited by the
MenB-4C vaccine we depleted anti-FHbp antibodies from pooled sera from the five  
macaques immunized with MenB-4C, and from the post-immunization serum of the  
immunized human. By ELISA the depletion removed >99% of the serum anti-FHbp  
antibodies but had no significant effect on serum IgG antibody titers to NadA, NHba or  
OMV (Fig. 4).

Despite high expression of NHba by the isolates from the University A outbreak,  
and high NHba and moderate NadA expression by the isolates from the University B  
outbreak, depletion of anti-FHbp antibodies from both the post-immunization macaque  
and human sera removed all or nearly all of the vaccine-induced bactericidal activity  
(Fig. 5, Panels A and C). In contrast, depletion of anti-FHbp antibodies had no effect  
on serum anti-PorA bactericidal activity against a control strain SK016 (Fig. 5, Panels B  
and D), which was mismatched for all of the antigens in MenB-4C except PorA P1.4.

Discussion

This study investigated susceptibility of serogroup B case isolates from two  
meningococcal outbreaks on U.S. university campuses to serum bactericidal antibody  
elicited by the MenB-4C vaccine. Our most important finding was that the isolates from  
both outbreaks were susceptible to vaccine-induced serum bactericidal activity.  
However, the isolates from the University B outbreak were more susceptible than those  
from the University A outbreak. Further, for both outbreaks, despite strain expression of  
two or three antigens that matched antigens in the MenB-4C vaccine, the major target  
of the vaccine-induced serum bactericidal activity was FHbp.
FHbp can be classified into three variant groups (23) or two sub-families (24) based on amino acid sequence similarities. In North America and Europe, approximately 50 to 60% of serogroup B case isolates have sub-family B FHbp (variant group 1), with the remaining isolates having sub-family A FHbp (variant groups 2 or 3) (24, 25). Considerable data indicate that FHbp vaccines elicit complement-mediated serum bactericidal activity primarily against strains that express a FHbp sequence variant from the same sub-family or variant group as the vaccine antigen (23, 26-28).

Although the isolates from both university outbreaks expressed sub-family B FHbp, which matched the sub-family B FHbp antigen in MenB-4C FHbp, the University B isolates were much more susceptible to anti-FHbp bactericidal activity than the University A isolates.

Previous studies identified several factors that can affect anti-FHbp bactericidal activity, even when there is a “match” between the vaccine FHbp sub-family and the sub-family of the strain. The most important are the extent of amino acid identity between the strain FHbp variant and vaccine antigen (16, 22, 29), together with the level of strain FHbp expression (5, 7, 30, 31). Additionally, the ability of meningococci to bind human FH using alternative ligands such as NspA (32, 33) or PorB (34, 35) can increase resistance to anti-FHbp bactericidal activity (34) (by FH down-regulation of complement activation). It is noteworthy that the more susceptible isolates from the University B outbreak expressed FHbp ID 1, which is an exact match to FHbp in MenB-4C vaccine, whereas the less susceptible isolates from University A expressed FHbp ID 276, with 96% amino acid identity to ID 1. In immunized mice, even this small difference in amino acid sequence can have a large effect on anti-FHbp bactericidal...
activity depending upon the locations of the amino acid residues (29). Further, while the isolates from both outbreaks were high expressers of FHbp, the University B isolates had greater surface-accessible FHbp detected by flow cytometry than the isolates from University A. Thus, the greater anti-FHbp bactericidal titers against the University B isolates likely resulted from greater expression of a FHbp that exactly matched the variant present in the vaccine. However, we cannot rule out a role for other factors that might have rendered the isolates from the University A outbreak more resistant to complement-mediated bactericidal activity, or the isolates from the University B outbreak more susceptible.

We anticipated that NHba (36-38), which was abundantly expressed by the isolates from both outbreaks, would elicit serum bactericidal antibodies (5). However, the isolates from both outbreaks were resistant to bactericidal activity of mouse anti-NHba antiserum. Further, depletion of anti-FHbp antibody from post-immunization sera from the MenB-4C-vaccinated macaques removed all of the bactericidal activity against the isolates from University A, and most of the bactericidal activity against isolates from University B. Depletion of anti-FHbp antibodies from the post dose 3-immunization human serum decreased bactericidal titers against both University A and B isolates to those obtained before dose 3 (titers of approximately 1:10, Figure 5). This subject had received two MenB-4C doses five years earlier and was a microbiologist working with N. meningitidis on nearly a daily basis. Thus, the low levels of serum bactericidal activity before dose 3 may have arisen from the earlier vaccination, or from naturally acquired antibodies, or from inadvertent laboratory exposure despite working in a BL2+ facility.
In our previous study, depletion of either anti-FHbp or anti-NHBA antibodies from sera of adults who had been immunized with the MenB-3C vaccine (i.e., similar to the MenB-4C vaccine but without the OMV component) resulted in loss of serum bactericidal activity against test strains mismatched for all of the MenB-4C antigens except NHba (10). We interpreted these results as showing “cooperative” bactericidal activity from vaccine-induced antibodies to NHba and cross-reacting antibodies to sub-family A FHbp, which individually were not bactericidal. In the present study, the isolates from both outbreaks had sub-family B FHbp, which matched the sub-family of FHbp in the MenB-4C vaccine. While we did not deplete anti-NHba antibody, the data from testing the antisera from mice immunized with individual antigens showed that sub-family B anti-FHbp antibodies alone were sufficient for bactericidal activity while the isolates were resistant to anti-NHba bactericidal activity. The basis for the resistance of the isolates from both university outbreaks to bactericidal activity by the mouse anti-NHba antiserum, and the post-immunization sera from macaques or human immunized with MenB-4C, which had been depleted to remove anti-FHbp antibodies, is unknown and will require further investigation.

Preliminary data presented at the June, 2015 meeting of the U.S. Advisory Committee on Immunization Practices are consistent with the present findings showing that the isolates from the University A outbreak are relatively resistant to serum bactericidal activity elicited by MenB-4C vaccination (http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2015-06/mening-03-macneil.pdf). Among 599 students attending University A and given two doses of the MenB-4C vaccine, only two-thirds had bactericidal titers of 1:4 or greater (the threshold
required for protection) in sera obtained two to four months after vaccination, with a reciprocal geometric mean titer of only 7.8. Thus, despite high expression of two vaccine antigens, FHbp and NHba, coverage against the isolates from the University A outbreak was incomplete within a few months after the students received two doses of the vaccine.

Measurement of antigen expression and/or cross-reactivity by a "meningococcal antigen typing system (MATS)" has been used extensively to predict MenB-4C vaccine coverage (39-43). Further the results have reported to be a conservative predictor of coverage (44). However, the results of the preliminary analysis of the serum bactericidal antibody responses of the vaccinated students from University A, together with the present data showing large differences in susceptibility to MenB-4C-induced bactericidal activity by isolates from the two college outbreaks, underscore that our understanding of the factors affecting strain susceptibility to bactericidal activity remain incomplete. As a result prediction of vaccine strain coverage by antibodies to different MenB-4C antigens will require additional study.

Acknowledgments

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We are grateful to the Meningitis Laboratory, the Meningitis and Vaccine Preventable Diseases Branch, Centers for Disease Control and Prevention (CDC), Atlanta, GA, for providing the isolates from both outbreaks and for the information on PorB and PorA, FHbp, NadA and NHba vaccine variants inferred from DNA sequencing studies. The isolates were provided to CDC by the California and New Jersey Departments of Health.

References


representative epidemiological meningococcal serogroup B panel confirms that MATS underestimates 4CMenB vaccine strain coverage. Vaccine 31:4968-74.

Table 1. Summary of control meningococcal strains

<table>
<thead>
<tr>
<th>Meningococcal Strain (Vaccine Antigen)</th>
<th>ST Clonal Complex</th>
<th>MenB-4C Vaccine Antigens</th>
<th>Antigen Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FHbp ID (Sub-family)</td>
<td>PorA VR1,2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NadA</td>
</tr>
<tr>
<td>NZ98/254 (FHbp and PorA)</td>
<td>41/44</td>
<td>14 (B)</td>
<td>1.7-2,4</td>
</tr>
<tr>
<td>SK016 (PorA)</td>
<td>103</td>
<td>25 (A)</td>
<td>1.7-2,4</td>
</tr>
<tr>
<td>H44/76 (FHbp)</td>
<td>32</td>
<td>1 (B)</td>
<td>1.7,16</td>
</tr>
<tr>
<td>5/99 (NadA)</td>
<td>8</td>
<td>23 (A)</td>
<td>1.5,2</td>
</tr>
<tr>
<td>M4407 (NHba)</td>
<td>41/44</td>
<td>19 (A)</td>
<td>1.19,15-1</td>
</tr>
</tbody>
</table>

- The antigens present in the MenB-4C vaccine are NadA, sub-family B FHbp, NHba and PorA P1.4 (in the OMV). Strain NZ98/254 expresses FHbp sub-family B and PorA 1.4. The remaining four control strains are each matched for only one of the four vaccine antigens known to elicit serum bactericidal activity.
- Clonal complex as defined by multi-locus sequence type (ST) (45).
- The MenB-4C vaccine contains sub-family B FHbp ID 1. FHbp peptide ID numbers and sub-family groups are from the FHbp database at http://pubmlst.org/neisseria/fHbp.
- PorA variable region (VR) 1 and 2 inferred from gene sequences. The MenB-4C vaccine contains OMV from strain NZ98/254, which contains PorA P1.7-2,4.
- Relative levels of antigen expression, +/-, +, ++ and +++ as measured by flow cytometry using live bacteria (see results). “Absent” indicates that the NadA gene is not present.
Table 2. Characteristics of invasive isolates from two university campus outbreaks

<table>
<thead>
<tr>
<th>Strain No.</th>
<th>University</th>
<th>ST Clonal Complex</th>
<th>MenB-4C Vaccine Antigen (% identity)</th>
<th>Sub-family B FHbp ID&lt;sup&gt;c&lt;/sup&gt;</th>
<th>PorA VR1,2&lt;sup&gt;d&lt;/sup&gt;</th>
<th>NadA&lt;sup&gt;e&lt;/sup&gt;</th>
<th>NHba&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH819</td>
<td>A</td>
<td>41/44</td>
<td>276 (96)</td>
<td>1.5,2-2</td>
<td>Absent</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>CH827</td>
<td>A</td>
<td>41/44</td>
<td>276 (96)</td>
<td>1.5,2-2</td>
<td>Absent</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>CH838</td>
<td>B</td>
<td>32</td>
<td>1 (100)</td>
<td>1.7,16-20</td>
<td>1.1 (95)</td>
<td>5 (91)</td>
<td></td>
</tr>
<tr>
<td>CH840</td>
<td>B</td>
<td>32</td>
<td>1 (100)</td>
<td>1.7,16-20</td>
<td>1.1 (95)</td>
<td>5 (91)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Two representative blood isolates were selected from each outbreak (see Methods).

<sup>b</sup> Clonal complex as defined by multi-locus sequence type (ST) (45).

<sup>c</sup> The MenB-4C vaccine contains sub-family B FHbp ID 1. The percent identity between the vaccine antigen and strain antigens are given in parentheses. From FHbp database at [http://pubmlst.org/neisseria/fHbp](http://pubmlst.org/neisseria/fHbp)

<sup>d</sup> Variable region (VR) type; the MenB-4C vaccine contains 1.7-2,4 (as the OMV antigen). The isolates from both outbreaks are mismatched to the PorA antigen in the vaccine.

<sup>e</sup> The MenB-4C vaccine contains NadA “peptide” 8 in variant group 2/3. The percent identity between the vaccine NadA antigen and strain antigen are given in parentheses. Antibodies to the vaccine antigen 2/3 are reported to cover strains with NadA-1 (20).

<sup>f</sup> The MenB-4C vaccine contains NHba variant 2. The percent identity between the vaccine antigen and strain antigens are given in parentheses.
FIG. 1. Vaccine antigen surface expression by isolates from the two outbreaks.

Antigen expression was measured by flow cytometry using live bacteria and mouse mAbs or antisera to each of the individual antigens (see Methods). Panel A, anti-capsular mAb. Isolate CH819 from University A, dashed line; Isolate CH840 from University B, solid black line; and a control strain NZ98/254, dotted line. Data for the three strains are superimposed. CH819 bacteria without added antibody, light gray shaded area (similar results without added antibody for the other two isolates, data not shown). Panel B, anti-FHbp mAb (JAR 41, which recognizes all FHbp sequence variants tested to date (14)). Line styles are the same as in Panel A. Panel C, mouse anti-NHba antiserum. Line styles are the same as in Panel A except the control strain is M4407, which is a naturally high expresser of NHba (10). Panel D, mouse anti-NadA antiserum. Line styles are the same as in Panel A except the control strain is 5/99, which is a naturally high expresser of NadA (9, 11).

FIG. 2. Bactericidal activity of antisera from mice immunized with individual antigens. Data are shown for one representative isolate from each of the University outbreaks (CH819 and CH840, Table 2) and four control strains (Table 1), each matched for only one of the four antigens in MenB-4C reported to elicit bactericidal activity: strain H44/76 (FHbp), 5/99 (NadA), M4407 (NHba) and SK016 (PorA P1.4) (Table 1). The dilution of the anti-PorA P1.4 mAb that killed strain SK016 is arbitrary; the strain was killed by <0.4 µg/ml. The outbreak strains from University A and University B were only killed by the anti-FHbp antiserum. The data are reported as mean ± range of two independent experiments. Similar respective results were obtained.
with two other outbreak isolates tested, CH827 and CH838 (University A and B, respectively; data not shown).

**FIG. 3. Serum bactericidal antibody responses.** Percent survival of strain CH819 (University A, open circles) or strain CH840 (University B, filled squares) when incubated for 60 min with test sera and 20% human complement. **Panel A.** Data shown for 1:10 dilution of individual sera from negative control mice immunized with aluminum hydroxide alone (~100% survival, no killing). **Panel B.** Sera from mice immunized with a recombinant sub-family B FHbp ID 1 (antigenic variant in MenB-4C vaccine). Mean percent survival ± SE of three serum pools tested at different dilutions (4 mouse sera per pool; 2 to 3 replicate assays). **Panel C.** Sera from three negative control unvaccinated macaques (mean survival at different dilutions). **Panel D.** Post-immunization sera from five macaques immunized with MenB-4C vaccine (mean percent survival at different dilutions tested twice). **Panel E.** Serum from a healthy adult human obtained two weeks before a third dose of MenB-4C given five years after dose 2. **Panel F.** Serum from the adult human obtained 6 weeks after dose 3 (mean from four replicate values).

**FIG. 4. Depletion of serum anti-FHbp antibodies.** A post-dose 2 immunization serum pool from five rhesus macaques vaccinated with MenB-4C, and a post-dose 3 immunization serum from an immunized human, were depleted of anti-FHbp antibodies by incubation with FHbp coupled with Sepharose (see Methods). The untreated sera, the FHbp-depleted sera and the mock-adsorbed sera were tested by ELISA for IgG antibodies to FHbp, NadA, NHba and OMV. The FHbp depletion removed >99% of the
anti-FHbp antibodies but had no significant effect on the serum antibody titers to NadA, NHba or OMV.

**FIG 5. Effect of depletion of serum anti-FHbp antibody on bactericidal activity.**

Panel A. Bactericidal activity of macaque serum depleted of anti-FHbp antibody (FHbp) against isolate CH819 (University A) or CH840 (University B). Serum pools from unvaccinated animals (N=3) and animals immunized with two doses of MenB-4C vaccine (Post-D2, N=5) were obtained as part of a previous study (16). Serum pools were depleted using an FHbp-Sepharose column or a non-conjugated Sepharose column as a control (Mock) (see Methods). Reciprocal mean bactericidal titers with range from two replicate assays are shown. Panel B. Macaque serum bactericidal activity measured against control strain SK016 (mismatched for all of the MenB-4C antigens except PorA P1.4 (8)). Panel C. Human serum from a subject immunized with MenB-4C five years after two previous doses (18). “Pre-D3” refers to serum obtained 2 weeks before dose 3 (approximately 5 years after doses 1 and 2). The post-dose 3 serum was obtained 3 weeks after vaccination. Panel D. Human serum bactericidal activity measured against control strain SK016 (mismatched for all of the MenB-4C antigens except PorA P1.4 (8)).