Prevalence of Factor H–Binding Protein Variants and NadA among Meningococcal Group B Isolates from the United States: Implications for the Development of a Multicomponent Group B Vaccine

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Background. Two promising recombinant meningococcal protein vaccines are in development. One contains factor H–binding protein (fHBP) variants (v.) 1 and 2, whereas the other contains v.1 and 4 other antigens discovered by genome mining (5 component [5C]). Antibodies against fHBP are bactericidal against strains within a variant group. There are limited data on the prevalence of strains expressing different fHBP variants in the United States.

Methods. A total of 143 group B isolates from patients hospitalized in the United States were tested for fHBP variant by quantitative polymerase chain reaction, for reactivity with 6 anti-fHBP monoclonal antibodies (MAb) by dot immunoblotting, and for susceptibility to bactericidal activity of mouse antisera.

Results. fHBP v.1 isolates predominated in California (83%), whereas isolates expressing v.1 (53%) or v.2 (42%) were common in 9 other states. Isolates representative of 5 anti-fHBP MAb–binding phenotypes (70% of isolates) were highly susceptible to anti–fHBP v.1 or v.2 bactericidal activity, whereas 3 phenotypes were ∼50% susceptible. Collectively, antibodies against the fHBP v.1 and v.2 vaccine and the 5C vaccine killed 76% and 83% of isolates, respectively.

Conclusions. Susceptibility to bactericidal activity can be predicted, in part, on the basis of fHBP phenotypes. Both vaccines have the potential to prevent most group B disease in the United States.

Neisseria meningitidis is an important cause of bacterial sepsis and meningitis worldwide. Polysaccharide-protein conjugate vaccines have been developed to protect against disease caused by strains with capsular groups A, C, W-135, or Y [1–4]. However, group B isolates, for which there is currently no broadly protective vaccine, accounted for 36%–49% of meningococcal isolates in the Active Bacterial Core Surveillance Reports of the Centers for Disease Control and Prevention from 2002 to 2005 (available at: http://www.cdc.gov/ncidod/dbmd/abcs/survreports.htm) and an even higher proportion of disease-producing isolates in Europe [5]. The group B polysaccharide is a self-antigen [6–8].
Therefore, most recent investigations of group B vaccine candidates have focused on noncapsular antigens [9–11], because there are safety issues for a capsular-based vaccine that could elicit autoantibodies.

A number of recombinant protein antigens have been investigated. These include proteins that are abundant in the outer membrane [12] (e.g., PorA [13, 14] and PorB [13]) or that are highly conserved, such as NspA [15]. Also, proteins that are not necessarily abundant but that have been identified by genomic studies and are predicted to be surface accessible and conserved in Neisseria species are being investigated [16, 17]. Some of these appear to be promising vaccine candidates in light of their ability to elicit serum bactericidal antibody against genetically diverse strains in mice [18–20]. These studies led to the development of an experimental, 5-component (5C), recombinant protein vaccine [21] that is currently being evaluated in humans [22].

The 5C vaccine contains 2 recombinant fusion proteins (genome-derived neisserial antigen [GNA] 2132-1030 and GNA 2091-1870) and 1 individual recombinant protein, NadA. The most potent immunogen in the 5C vaccine appears to be GNA 1870, which is also known as “lipoprotein 2086” [23]. This protein recently has been renamed “factor H–binding protein” (fHBP) [24], to reflect the discovery that one of its functions is to bind factor H (fH), an important complement down-regulatory protein (see below) [25–27]. A second recombinant-protein vaccine that contains both the fHBP variant (v.) 1 and 2 proteins also is under development.

In a previous study, mouse antiserum raised against the 5C recombinant-protein vaccine was bactericidal against 78%–98% of a panel of 85 strains, depending on the adjuvant used in the vaccine formulation [21]. However, rabbit complement was used for measurement of bactericidal activity, which is known to greatly augment the susceptibility of N. meningitidis to bacteriolysis [28, 29]. Also, N. meningitidis recently has been shown to bind fH [24, 30], which provides a novel mechanism by which the organism can inactivate specific complement components and evade complement-mediated killing. Binding is specific for human fH [31]. Lack of binding of rabbit fH may explain why N. meningitidis is more susceptible to killing by rabbit complement than by human complement. Therefore, serum bactericidal susceptibility data generated with rabbit complement may not be reliable for predicting the potential efficacy of a group B vaccine, particularly one that contains fHBP as one of its principal antigens.

In the present study, we analyzed the prevalence of fHBP variants in 3 collections of disease-producing group B meningococcal isolates from different regions of the United States. We also tested the susceptibility of the isolates to human complement–mediated killing by mouse antisera prepared against each of the individual fHBP variants and the 5C vaccine. The data permit estimates of the maximum potential strain coverage in different regions of the United States by the 5C vaccine containing fHBP v.1 as one of its antigens and by a 2-component vaccine containing fHBP v.1 and v.2.

**METHODS**

**Meningococcal isolate collections.** Group B meningococci (n = 144) were obtained from 3 collections. The California collection consisted of consecutive isolates referred to the California Department of Health in 2003 and 2004. These isolates were from 48 patients of a variety of ages hospitalized in 22 counties. The Maryland collection was from cases of meningococcal disease in residents of Maryland of a variety of ages who were hospitalized between 1995 and 2005 (n = 50). These isolates were collected as part of the Maryland Active Bacterial Core Surveillance project [32]. The third collection was from a prospective, multicenter surveillance study of meningococcal disease in children (age 0–16 years) who were admitted to 10 pediatric hospitals in 9 US states between 2001 and 2005 (n = 46) [33].

The multicenter collection included 8 isolates from 2 hospitals in California. To avoid potential overlap between these isolates and those in the California collection, we obtained information on the dates of isolation and compared respective sequence types (STs), quantitative polymerase chain reaction (QPCR) results, and fHBP monoclonal antibody (MAb) reactivity. We identified 1 duplicated isolate, which was omitted from the data analysis, leaving 143 isolates. In addition, for purposes of data analysis, the 7 isolates from the multicenter collection that originated in California were grouped with the California collection.

**Growth and preparation of meningococcal strains.** N. meningitidis strains were cultured, and heat-killed cells were prepared for QPCR and for dot immunoblotting as described elsewhere [34].

**Identification of alleles by QPCR.** Grouping of fHBP variants was performed by QPCR as described elsewhere [34]. We used QPCR to determine whether the nadA gene was present by use of the specific primers NadA IF (5′-AACCTTACACGTCGGTCCGTTCA-3′) and NadA IR (5′-ACTCGTAAATGGACGCGCACATGTT-3′) under reaction conditions that have been described elsewhere [34]. A positive signal was indicated by a cycle threshold (C) value between 16.6 and 26.9, and a negative signal was indicated by a C value between 28.3 and 40.0. Amplification of the 16S rRNA gene [34] was performed to account for differences in the levels of template DNA. The difference between the 16S result and a positive fHBP or nadA signal was <5.8 C; for a negative signal, it was >10.9 C.

**Preparation of MABs.** MABs against recombinant fHBP v.1 (from strain MC58)–JAR 1, 3, and 5—have been described elsewhere [35]. MABs against recombinant fHBP v.2 (from
strain 2996)—JAR 10, 11, and 13—were made using a similar procedure. As described below, JAR 10 cross-reacts with a subset of strains that express fHBP v.1, and JAR 13 cross-reacts with some v.3 strains.

Detection of proteins by dot immunoblotting. Heat-killed cell suspensions (~1 × 10^8 cells in 100 μL) were applied to nitrocellulose membranes (Bio-Rad) under vacuum. The primary antibody was either a MAb (1–10 μg/mL) or an appropriate dilution (1:5000 to 1:10,000) of mouse polyclonal antiserum to fHBP v.1, v.2, and v.3 or to NadA. The secondary antibody was a 1:10,000 dilution of rabbit anti–mouse IgG/IgM–horseradish peroxidase conjugate (Invitrogen). The antibody was a 1:10,000 dilution of rabbit anti–mouse IgG/ IgM–horseradish peroxidase conjugate (Invitrogen). The membranes were developed with Western Lightning chemiluminescent substrate (Perkin-Elmer) and exposed to X-OMAT film (Eastman Kodak).

Bactericidal assays. Bactericidal assays were performed as described elsewhere using mid-log-phase bacteria grown in Mueller-Hinton broth to an OD620 of ~0.6 [36, 37]. The complement source was human serum that was characterized as described elsewhere [37]. The buffer was Dulbecco’s PBS with Ca^{2+} and Mg^{2+} (Mediatech) containing 1% (wt/vol) bovine serum albumin (Sigma-Aldrich). The antisera against the fHBP v.1, 2, or 3 protein [19]; GNA 2132 [16, 20]; NadA [18]; or the 5C vaccine [21] were prepared in mice by use of complete and incomplete Freund’s adjuvant (Sigma-Aldrich). Assays of selected NadA-positive isolates were repeated using late-log-phase bacteria grown to the same optical density (0.8–0.9) [18].

RESULTS

STs, fHBP gene variants, and nadA presence. Table 1 summarizes the distribution of STs among the isolates. Thirty-one percent of the isolates from patients in California had identical respective ST, porA, porB, and fetA genotypes, which were consistent with a specific clonal group. Isolates with this genotype were less prevalent in the other 2 collections (~6%). With the exception of isolates from 3 patients hospitalized during a 2-week period in 1 county, the remaining California isolates with this genotype came from patients hospitalized at different times of the year and/or in different counties. Therefore, as a group the isolates were not part of a specific outbreak.

QPCR results showed that the fHBP gene was present in all 143 isolates. Collectively, 65% were fHBP v.1, 31% were v.2, and 4% were v.3. As shown in figure 1A, the fHBP v.1 gene predominated in isolates from California (83% v.1, 13% v.2, and 4% v.3), whereas the Maryland collection had 52%, 44%, and 4% and the multicenter collection had 57%, 37%, and 4%, respectively. The respective percentages in the latter 2 collections were similar to each other and were collectively different from those in the California collection (P<.01, χ² test). In all, 71 isolates (50%) were nadA positive; 77% of the California isolates were positive for the nadA gene, compared with 32% and 37%, respectively, in the Maryland and multicenter collections (P<.001) (figure 1B). All of the ST-32 isolates with identical respective porA, porB, and fetA genotypes (table 1) were positive for the fHBP v.1 and nadA genes. If these isolates are removed from the analysis of the frequencies of fHBP variants and nadA, the respective proportions in the 3 collections are still significantly different (P<.02).

Reactivity with anti-fHBP and anti-NadA antibodies. Ninety-seven percent of isolates expressed fHBP, as detected by dot immunoblotting using polyclonal antiserum prepared against fHBP v.1, v.2, and v.3. Of those with detectable expression of fHBP, there was moderate (up to 10-fold) variation in the quantity expressed by the different isolates. Of 71 isolates with the nadA gene, as detected by QPCR, 99% expressed the NadA protein, as detected by dot immunoblotting with polyclonal anti-NadA antisera. NadA expression in cultures grown to the same optical density (0.8–0.9) was estimated to differ by as much as 100-fold. All of the isolates that were

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Table 1. Distribution of sequence types (STs) among group B meningococcal isolates.

<table>
<thead>
<tr>
<th>ST complex (lineage)</th>
<th>California (n = 55)</th>
<th>Maryland (n = 50)</th>
<th>Multicenter* (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-32, clonalb (ET-5)</td>
<td>31</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>ST-32, otherc (ET-5)</td>
<td>33</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>ST-41/44 (lineage III)</td>
<td>13</td>
<td>26</td>
<td>34</td>
</tr>
<tr>
<td>ST-162</td>
<td>6</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>ST-269</td>
<td>0</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Otherd</td>
<td>17</td>
<td>18</td>
<td>26</td>
</tr>
</tbody>
</table>

* Seven isolates from the multicenter collection originated in California and are grouped with the California isolates (see Methods).

b Defined as ST-32; porA VR1 and VR2 of 7,16-20; and porB of 3-24 and fetA of 3-3.

c ST-32 isolates with combinations of porA, porB, and fetA genotypes different from that of the clonal group.

d No single ST complex comprised >5% of isolates in this category.

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Figure 1. Percentage of fHBP variant (v.) 1, 2, or 3 genes (A) and nadA genes (B), as determined by quantitative polymerase chain reaction. Error bars indicate 95% confidence intervals. Shaded bars, California isolates; hatched bars, Maryland isolates; white bars, multicenter isolates.
negative for the \textit{nadA} gene by QPCR also were negative by dot immunoblotting.

The 6 MAbS made against fHBP v.1 or v.2 differed in their reactivity on whole-cell dot immunoblots with a panel of control strains and, therefore, appear to recognize unique epitopes. The MC58 wild-type (WT) strain (fHBP v.1) was positive by dot immunoblotting for reactivity with MAbS JAR 1, 3, and 5 (figure 2), which were prepared against the recombinant fHBP v.1 expressed from the gene from MC58 \cite{35}. In contrast, the MC58 WT strain did not react with MAbS JAR 10, 11, or 13, which were prepared against the recombinant fHBP v.2 expressed from the gene from strain 2996 (figure 2). None of the 6 MAbS bound to the MC58 fHBP knockout (KO) strain. Group B strain NZ98/254, which expresses a v.1 fHBP that is 91.3\% identical to that of MC58 \cite{35}, was recognized by 2 of the anti-fHBP v.1 MAbS, JAR 3 and 5, as well as by the anti-v.2 MAb JAR 10. The 8047 WT strain, which expresses a v.2 fHBP, was recognized by all 3 of the anti-v.2 MAbS but by none of the anti-v.1 MAbS (figure 2). The M1239 WT strain, which expresses a v.3 fHBP, was recognized only by the anti-v.2 MAb JAR 13. The respective patterns of MAb reactivity can be used to describe different fHBP phenotypes. We established a binary system of nomenclature to indicate the pattern of reactivity of the fHBP MAbS in the order JAR 1, 3, and 5 (anti-v.1) followed by JAR 10, 11, and 13 (anti-v.2), with 1 indicating positive reactivity and 0 indicating no reactivity. Thus, the fHBP phenotype of the v.1 strain MC58 is 111-000, and that of the v.2 strain 8047 is 000-111.

Next, we examined the fHBP MAb reactivity of the 143 isolates in the 3 collections. As summarized in table 2, among the 93 isolates expressing fHBP v.1, phenotype 111-000 was the most common (70\% of v.1 isolates). Isolates with this fHBP phenotype were predominantly electrophoretic type (ET) –5 (89\%), ST –32 (71\%), and \textit{nadA} positive (83\%). Two other phenotypes, 011-000 and 011-100, constituted 9\% and 10\% of the v.1 isolates, respectively. These groups had heterogeneous STs, and only 11\% and 38\%, respectively, were \textit{nadA} positive (P<.0001, compared with the proportion of \textit{nadA}-positive 111-000 isolates). Other fHBP phenotypes (111-100, 001-100, and 000-000) collectively accounted for the remaining 11\% of the v.1 isolates.

fHBP in the v.2 group have, on average, 85\% aa identity with the v.3 group, whereas both of these groups have lower homology with fHBPs in the v.1 group (74 and 63\%, respectively) \cite{19}. There also is considerable cross-reactivity between v.2 and v.3 proteins but not between either of these and v.1 proteins (see below). Therefore, for the purpose of analysis of the fHBP phenotypes, the data from strains expressing v.2 or v.3 proteins were combined. Among the 50 fHBP v.2 or v.3 (v.2/v.3) isolates, phenotype 000-111 occurred in 20\%; this group contained both v.2 and v.3 isolates, and these were predominantly in the ST-35 complex. Phenotype 000-110 occurred in 24\% of the v.2/v.3 isolates, and these isolates were all v.2 and were predominantly lineage III. Phenotype 000-001, which is typified by the fHBP v.3 strain M1239, occurred in 12\% of the v.2/v.3 isolates; this group contained both v.2 and v.3 isolates, and these were primarily in the ST-162 complex. Isolates with the phenotype 000-000, which do not react with any of the MAbS, comprised 44\% of the v.2/v.3 isolates. All of these isolates expressed v.2 proteins. They had diverse STs and likely represent a genetically heterogeneous collection.

\textbf{Bactericidal activity of anti-fHBP antisera.} We measured

\begin{table}[h!]
\centering
\begin{tabular}{|c|c|c||c|c|}
\hline
fHBP phenotype & fHBP variant & Isolates & Prototype strain & Predominant ST (ET) complex \\
\hline
\hline
111-000 & 1 & 1 & 70 & MC58 & ST32 (ET5) \\
011-000 & 1 & 1 & 9 & 4243 & Heterogeneous \hline
011-100 & 1 & 1 & 10 & NZ98/254 & Heterogeneous \hline
Other v.1 & 1 & 1 & 11 & None & None \hline
000-111 & 2 or 3 & 20 & 2996 & ST35 complex \hline
000-110 & 2 or 3 & 24 & MD01158 & ST41/44 (lineage III) \hline
000-001 & 2 or 3 & 12 & M1239 & ST162 complex \hline
000-000 & 2 & 44 & RM1090 & Heterogeneous \hline
\end{tabular}
\caption{Prevalence of factor H–binding protein (fHBP) isolates, by fHBP phenotype.}
\end{table}

\textit{a} Pattern of fHBP monoclonal antibody (MAb) reactivity in the order JAR 1, 3, 5 (anti–variant \textit{v.1} MAbS) and JAR 10, 11, 13 (anti–variant \textit{v.2} MAbS); 1 indicates reactivity, and 0 indicates no reactivity.

\textit{b} Among \textit{v.1} or among \textit{v.2} and \textit{v.3} isolates, respectively.

\textit{c} Multilocus sequence type (ST), per the Multi Locus Sequence Typing database (available at: http://www.mlst.net) \cite{38}. “Predominant” implies that >50\% of isolates had the specified ST.
the bactericidal activity of anti-fHBP v.1, v.2, and v.3 antisera on 84 of the isolates (48 v.1; 36 v.2; and 3 v.3). The anti-v.1 antiserum was bactericidal (titer $\geq 1:8$, with human complement) against 77% of the v.1 isolates, whereas the anti-v.2 and anti-v.3 antisera killed only 2% and 0%, respectively, of the v.1 isolates. v.1 isolates representative of phenotypes 111-000 and 011-000 were particularly susceptible to the bactericidal activity of the anti-v.1 antiserum (91% and 100% of isolates, respectively), whereas only 50% of isolates with phenotype 011-100 or with other v.1 phenotypes were susceptible to killing by this serum (figure 3A). Together, these latter groups comprised 21% of v.1 isolates (table 2). The respective geometric mean titers (GMTs) exhibited similar patterns. The GMTs against v.1 isolates with either the 111-000 or 011-000 phenotype were $\geq 1:600$, whereas those against isolates with the remaining phenotypes were between 1:30 and 1:50 (figure 3B).

The majority (74%) of the v.2/v.3 isolates were susceptible to the bactericidal activity of the anti-v.2 antiserum. Of the isolates with a 000-111, 000-110, or 000-001 phenotype, 78%–100% were susceptible, whereas only 46% of the isolates with the 000-000 phenotype were susceptible (figure 4A). The GMTs of the anti-v.2 antiserum against isolates with 1 of the first 3 phenotypes were between 1:200 and 1:1500 and was 1:25 for the phenotype 000-000 (figure 4B). Most of the v.2/v.3 phenotypes showed similar results with the anti-v.3 antiserum. One exception was the 000-111 phenotype; of the isolates with this phenotype, 67% were susceptible to anti-v.3 antiserum (GMT, 1:70), versus 100% being susceptible to the anti-v.2 antiserum (GMT, 1:1500).

**Bactericidal activity of anti-5C antiserum.** Ninety-five percent of the 48 fHBP v.1 isolates tested were killed (titer $\geq 1:8$) by the polyclonal antiserum from mice immunized with the 5C vaccine (figure 5A), which contains fHBP v.1, compared with 56% of the v.2/v.3 isolates (figure 5B) ($P<.001$). Only 2 v.1 isolates were not killed by the anti-5C antiserum, and both were in the other-phenotype category. The GMTs of the anti-5C antiserum against v.1 isolates with different fHBP phenotypes ranged from 1:150 to 1:1500. The susceptibility of the v.2/v.3 isolates with different phenotypes ranged from 33% to

![Figure 3](https://academic.oup.com/jid/article-abstract/195/10/1472/2191860)

**Figure 3.** Bactericidal activity of anti-factor H–binding protein (fHBP) antisera against fHBP variant (v.) 1 isolates, by fHBP phenotype ($n = 8$–21 isolates/group). A, Percentage of strains giving a titer $\geq 1:8$; B, Reciprocal geometric mean titers (GMTs). Error bars indicate 95% confidence intervals. Shaded bars, anti–fHBP v.1 antiserum; hatched bars, anti–fHBP v.2; white bars, anti–fHBP v.3.

![Figure 4](https://academic.oup.com/jid/article-abstract/195/10/1472/2191860)

**Figure 4.** Bactericidal activity of anti-factor H–binding protein (fHBP) antiserum against isolates expressing fHBP variant (v.) 2 or 3. Each fHBP phenotype had 6–13 isolates. A, Percentage of strains giving a titer $\geq 1:8$; B, Reciprocal geometric mean titers (GMTs). Error bars indicate 95% confidence intervals. Shaded bars, anti–fHBP v.1 antiserum; hatched bars, anti–fHBP v.2; white bars, anti–fHBP v.3.
Figure 5. Percentage of isolates giving a titer ≥1:8 to anti–5-component (5C) vaccine antiserum. Also shown are data for the respective percentages susceptible to the anti–factor H–binding protein (fHBP) variant (v.) 1 antiserum tested alone. A, fHBP v.1 isolates; B, fHBP v.2 or v.3 isolates. Error bars indicate 95% confidence intervals. Stippled bars, anti-5C antiserum; shaded bars, anti-fHBP v.1.

62% (figure 5B), and no v.2/v.3 phenotype correlated strongly with susceptibility to antibodies elicited by the 5C vaccine. The respective GMTs of the v.2/v.3 isolates also were significantly lower (1:10–1:30) than those of the v.1 isolates, which may reflect the lack of bactericidal activity of anti-v.1 antibodies against v.2/v.3 isolates and the lower bactericidal activity of antibodies elicited by the other 4 antigens in the 5C vaccine.

**Contribution of GNA 2132 and NadA antigens in the 5C vaccine.** To assess the contributions of antigens other than fHBP v.1 in eliciting protective antibodies, we selected strains that were killed by the anti–5C antiserum but not by the anti-v.1 antiserum and tested their susceptibility to antisera prepared against 2 of the other antigens in the 5C vaccine, GNA 2132 [16, 20] and NadA [18]. Only 1 of 7 fHBP v.1 isolates tested was killed (titer ≥1:8) by the anti–GNA 2132 antiserum, compared with 8 of 10 fHBP v.2/v.3 isolates (P<.02, Fisher’s exact test). Five of the 10 isolates expressing fHBP v.2/v.3 had the nadA gene, and 1 of these was killed by the anti-NadA antiserum. Of the 7 v.1 isolates tested, the nadA gene was present in 3, and none were killed by the anti-NadA antiserum.

**DISCUSSION**

Among the 3 collections, 94% of isolates could be categorized into 7 patterns of anti-fHBP MAb reactivity, which we refer to as “fHBP phenotypes,” and some of the phenotypes predicted the susceptibility of an isolate to vaccine-induced serum bactericidal activity. For example, >90% of isolates with the 111-000 or 011-000 phenotype were highly susceptible to anti-v.1 antiserum, whereas only 50% of v.1 isolates with other phenotypes were susceptible (figure 3A). Similarly, 100% of v.2/v.3 isolates with the 000-111 or 000-001 phenotypes were susceptible to the bactericidal activity of the anti-v.2 antiserum, compared with 46% of v.2/v.3 isolates with the null phenotype (000-000). The most likely reasons for resistance of some of these isolates are genetic variability of the antigen and/or low expression of the protein [19].

Anti-5C complement–mediated killing of isolates expressing v.2 or v.3 fHBP appears to result from bactericidal anti-GNA 2132 antibodies and, to a lesser extent, anti-NadA antibodies. The anti-5C antibodies responsible for the killing of isolates expressing v.1 proteins that were not killed by anti-v.1 antiserum were not identified. Conceivably, these isolates might have been killed by antibodies against GNA 1030 or GNA 2091 or by a combination of antibodies elicited by the vaccine. However, the one combination that we tested (anti–fHBP v.1, anti-NadA, and anti–GNA 2132) had no bactericidal activity against these strains (data not shown).

NadA is known to play a role in the adhesion of *N. meningitidis* and invasion of human epithelial cells [18, 39]. Although nadA is present in the majority of the v.1 strains, the absence of nadA in some strains indicates that the presence of this molecule is not essential for pathogenesis. NadA can elicit serum bactericidal antibodies in mice against some NadA-positive strains [18], but its role in eliciting bactericidal antibodies by the 5C vaccine is difficult to define. First, the presence of the nadA gene segregates with the fHBP phenotype 111-000, which is the most common phenotype among v.1 isolates (70%; table 2), and the anti-v.1 antiserum alone had high bactericidal titers (>1:2000) against 90% of these isolates (figure 3). The main group of isolates for which anti-NadA antibodies might be important are the v.2/v.3 isolates that are nadA positive, but these constitute a small proportion of all isolates (6%). Nevertheless, antibodies against NadA can be bactericidal and may decrease colonization by strains expressing this protein. If true, the presence of NadA in the 5C vaccine could decrease the selection and emergence of mutant *N. meningitidis* strains that are resistant to the bactericidal activity of anti-v.1 antibodies.

The potential strain coverage for antibodies elicited by a vaccine containing single fHBP variants, multiple variants, or multiple antigens can be assessed by combining the prevalence of each phenotype with the proportion of isolates with each of
the phenotypes susceptible to bactericidal activity. The combination of fHBP v.1 and v.2, or the 5C vaccine containing the v.1 fHBP, is projected to elicit bactericidal activity against 76% and 83% of strains, respectively. These are maximum projections of strain coverage based on serum bactericidal titers of 1:8 or higher in mouse antisera assayed with human complement.

Recently, the 5C vaccine was investigated in a phase 1 trial conducted in adults [22]. After a third injection, >90% of the immunized subjects developed serum bactericidal titers of ≥1:4 against at least 1 group B strain representative of different hypervirulent lineages (for example, ST-32 [ET-5], ST-41/44 [lineage III], or ST-8 [cluster A4]). The presence of different vaccine-antigen variants and/or their expression does not always correlate with ST, and the percentages of immunized subjects with serum bactericidal activity against other test strains, some with STs identical to those of susceptible isolates, were lower. Nonetheless, this multicomponent vaccine is the first recombinant-protein vaccine to elicit serum bactericidal antibodies in humans against genetically diverse group B strains and, thus, appears to be promising. The data also demonstrate the power of the “reverse vaccinology” approach [40, 41] for the discovery of promising antigens.

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References


