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The Role of Anti-NHba Antibody in Bactericidal Activity Elicited by the Meningococcal Serogroup B Vaccine, MenB-4C

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Abstract

Background—MenB-4C (Bexsero®) is a multicomponent serogroup B meningococcal vaccine. For vaccine licensure, efficacy was inferred from serum bactericidal antibody (SBA) against three antigen-specific indicator strains. The bactericidal role of antibody to the fourth vaccine antigen, Neisserial Heparin binding antigen (NHba), is incompletely understood.

Methods—We identified nine adults immunized with two or three doses of MenB-4C who had sufficient volumes of sera and >3-fold increases in SBA titer against a strain with high NHba expression, which was mismatched with the other three MenB-4C antigens that elicit SBA. Using 1 month-post-immunization sera we measured the effect of depletion of anti-NHba and/or anti-Factor H binding protein (FHbp) antibodies on SBA.

Results—Against three strains matched with the vaccine only for NHba, depletion of anti-NHba decreased SBA titers by an average of 43–79% compared to mock-adsorbed sera ($P < 0.05$). Despite expression of sub-family A FHbp (mismatched with the subfamily B vaccine antigen), depletion of anti-FHbp antibodies also decreased SBA by 45–64% ($P < 0.05$). Depletion of both antibodies decreased SBA by 84–100%. Against a strain with sub-family B FHbp and expression of NHba with 100% identity to the vaccine antigen, depletion of anti-NHba decreased SBA by an average of 26%, compared to mock-adsorbed sera ($P < 0.0001$), and depletion of anti-FHbp antibody decreased SBA by 92% ($P < 0.0001$).

Conclusions—Anti-NHba antibody can contribute to SBA elicited by MenB-4C, particularly in concert with anti-FHbp antibody. However, some high NHba-expressing strains are resistant, even with an exact match between the amino acid sequence of the vaccine and strain antigens.

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Conflict of interest: DMG is an inventor on patent applications or on issued patents in the area of meningococcal vaccines. Rights to these inventions have been assigned to UCSF Benioff Children's Hospital Oakland. EP, EL, SG and DMV have declared that no conflicts of interest exist

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Keywords

Neisseria meningitidis; vaccine; Neisserial Heparin binding antigen; Factor H binding protein; FHbp; complement

Introduction

The multicomponent meningococcal serogroup B vaccine, MenB-4C (Bexsero®), was licensed in the U.S. in 2015. The vaccine contains four antigens known to elicit SBA: Factor H binding protein (FHbp), Neisserial heparin binding antigen (NHba), Neisserial adhesin A (NadA) and PorA P1.4 (in outer membrane vesicles, OMV) [1, 2]. Evidence indicates that antibodies elicited in humans to FHbp [3, 4], NadA [4, 5] and PorA serosubtype antigens [6, 7] have complement-mediated serum bactericidal activity. SBA is a widely accepted correlate of protection against developing invasive meningococcal disease [8, 9], and for vaccine licensure was the basis of inferring MenB-4C efficacy (<https://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm431374.htm>).

FHbp can be classified into two sub-families [10], A and B, or three variant groups, 1,2 and 3 [11]. In general, SBA elicited by FHbp is thought to be sub-family specific (i.e., antibodies elicited by FHbp antigens in sub-family A have SBA primarily against strains with FHbp from sub-family A but not sub-family B and vice versa) [10]. MenB-4C contains FHbp antigen from sub-family B (ID1) [1]. Approximately 40 to 50 percent of disease-causing strains in the U.S. have FHbp antigens from sub-family A [12, 13]. Protection elicited by MenB-4C against these strains is thought to depend largely on antibodies induced by the other three vaccine antigens [14].

The bactericidal role of serum antibodies to the fourth MenB-4C antigen, NHba, is incompletely understood. For example, during MenB-4C vaccine licensure in the United States, the manufacturer provided SBA against three “antigen-specific” indicator strains but was unable to provide SBA data for an anti-NHba indicator strain [14]. Furthermore, in MenB-4C-immunized infant rhesus macaques, and an immunized adult human, depletion of serum anti-FHbp antibody eliminated SBA against two meningococcal strains with high strain expression of NHba [15]. These results suggested that some strains are resistant to anti-NHba SBA. The purpose of the present study was to investigate the role of complement-mediated serum anti-NHba bactericidal activity elicited in adults immunized with MenB-4C using additional test strains selected for different antigenic compositions with respect to antigens in MenB-4C.

Methods

Serum samples

Twenty adults were immunized with MenB-4C in a previous study [5]. Eight (40%) had >3-fold SBA responses against strain M4407 when comparing titers of pre- and 1-month post dose 2 sera (Supplemental Table S1). Five additional subjects (25%) had >2-fold and 3-fold SBA responses, which likely represented true increases in SBA in response to vaccination since the pre- and post-dose 2 sera were assayed in parallel on more than one

occasion. We chose strain M4407 as an anti-NHba indicator strain because it is a relatively high expresser of NHba with 100% amino acid identity to the MenB-4C vaccine antigen and is mismatched for the other three vaccine antigens (Table 1). We chose subjects with >3-fold SBA responses for inclusion in the present study because the response was the minimum required to demonstrate an effect of specific antibody depletion on SBA. Of the 8 subjects with >3-fold responses post-dose 2, 5 were selected for inclusion in the present study based on having sufficient volumes of sera for the antibody-depletion experiments. Four additional laboratory workers who had been given an off-label third dose of MenB-4C by their physician or the hospital Employee Health department, also were included for a total of nine subjects. These four additional subjects had >3-fold SBA responses 1 month after dose 3 (Supplemental Table S2), had sufficient serum for our studies, and provided written informed consent for providing the serum samples under a protocol approved by the Institutional Review Board of UCSF Benioff Children's Hospital Oakland (protocol number 2016-024). The ages of the 9 subjects ranged from 21 to 44 years (mean = 26.5). Three were male and 6 were female.

Preparation of immunoadsorbent columns

Recombinant NHba was purified from *E. coli* B121 (DE3) transformed with pET21b containing a NHba gene from serogroup B strain NZ98/254 [16]. The amino acid sequence of the recombinant protein was 100% identical to the NHba sequence in MenB-4C. We also prepared recombinant FHbp (sub-family B) using a FHbp gene isolated from group B strain H44/76. The FHbp amino acid sequence was identical to the vaccine antigen ID 1 except for a single amino acid substitution (Ser for Arg at residue 41), which decreased binding to complement Factor H (FH) by >100-fold [17]. We used the mutant antigen to avoid adsorption of FH to the column, which might have interfered with anti-FHbp antibody depletion. The recombinant proteins were coupled to cyanogen bromide-activated sepharose 4B beads according to the manufacturer's directions (catalogue number 68987-32-6, Sigma, St. Louis, MO). As a negative control (mock column), we coupled bovine serum albumin to cyanogen bromide-activated sepharose 4B using the same methods.

Depletion of serum anti-NHba and anti-FHbp antibodies

We depleted anti-NHba and anti-FHbp antibodies from post-immunization serum, rather than pre-immunization serum, in order to investigate the role of vaccine-induced antibodies in protection. Serum samples were heated at 56°C for 30 minutes to inactivate endogenous complement. Aliquots (1 ml) were incubated for 1 hr at room temperature with mock-, NHba-, FHbp-, or combined NHba+FHbp-conjugated immunoadsorbents. The serum was aspirated and stored at -80°C for subsequent SBA assays. The columns were eluted with 5 column volumes of 0.1M glycine pH 2.7 to remove bound antibody followed by extensive washing with PBS between serum depletions.

Adequacy and specificity of antibody depletion

Serum antibody titers were measured by ELISA using a method previously described [18], and the following antigens: recombinant FHbp, NHba, NadA, detergent-extracted outer membrane vesicles (dOMV) from a mutant strain of NZ98/254 in which the gene for FHbp had been inactivated, and tetanus toxoid (NIBSC #02/232, Potters Bar, Hertfordshire

England). The amino acid sequences of the FHbp, NHba and NadA proteins were identical to the respective MenB-4C antigens. Serial 2-fold dilutions of sera were added to wells of the microtiter plates and incubated for four hours at room temperature. After washing, bound antibody was detected with alkaline phosphatase-conjugated goat anti-human IgG, Fc-specific (Jackson ImmunoResearch Laboratories, West Grove, PA) [18]. To determine adequacy of antibody depletion, we compared the respective reactivity (OD₄₀₅) of a fixed dilution of the antigen-depleted and mock-depleted serum.

Neisseria meningitidis strains (Table 1)

For purposes of our study, we defined a strain “mismatched” for an antigen contained in MenB-4C as follows: FHbp, a sequence variant assigned to Sub-family A; NadA, an absent gene (all of the strains except the NadA reference strain 5/99 lacked a gene for NadA and had no detectable binding by flow cytometry using a mouse polyclonal anti-NadA antiserum); PorA, a variable region sequence type other than 1.4; and NHba, low expression as measured by flow cytometry using antiserum from mice immunized with a recombinant NHba vaccine with an exact amino acid sequence to the NHba antigen in MenB-4C. Low expression of NHba by a test strain was scored as “+/-”, which represented <20% antibody binding compared to the reference M4407 strain with moderately high natural expression of the antigen. Two of the test strains, 5/99 and SK016, were used in previous studies as anti-NadA and anti-PorA P1.4 antigen-specific SBA indicator strains, respectively [4, 19]. Strains M4407, B2 and B9 were used to assess anti-NHba SBA based on moderate to high expression of NHba with 87 to 100% amino acid identity to the MenB-4C NHba vaccine antigen, and mismatched for the other three MenB-4C antigens known to elicit SBA (Table 1). Two strains from college outbreaks also were investigated. The Princeton University strain had FHbp sub-family B (FHbp ID 276 with 96% amino acid identity with the sub-family B ID 1 antigen in MenB-4C and positive expression by flow cytometry), and expressed NHba with 100% amino acid identity to NHba in MenB-4C [15]. The Rutgers University strain was mismatched for all four MenB-4C antigens (Table 1) [20].

Antigen expression

Strain antigen expression was measured by flow cytometry using live bacteria as previously described [16, 21]. FHbp was measured with a 1:500 dilution of polyclonal serum from mice immunized with recombinant FHbp ID 19 (subfamily A) or ID 1 (sub-family B). The test strain results were compared with expression by naturally high FHbp-expressing strains H44/76 (sub-family B) or M4407 (sub-family A), depending on the FHbp sub-family of the test isolate. NHba was measured with a 1:800 dilution of serum from mice immunized with recombinant NHba ID 2 (identical sequence to NHba in MenB-4C). The test strain results were compared with NHba expression by strain M4407, which has relatively high expression of NHba ID 2. Antigen expression by the test strain is reported as +/- (<20% compared to the positive control strain tested in parallel); + (20–99% expression), or ++ (100% expression).

Complement-mediated serum bactericidal activity

Details of the SBA method have been described [21, 22]. All data were freshly generated for the present study. The complement source was pooled sera from three healthy adults, which

had been depleted of IgG using a protein G column (HiTrap protein G; GE Life Sciences, Piscataway, NJ) [21]. SBA titers were defined by the serum dilution resulting in 50% survival of bacteria (CFU/ml) after incubation for 60 minutes compared to CFU/ml with negative control sera and complement.

Statistical analysis

In order to assess the role of vaccine-induced antibodies in protection, for calculation of the effect of depleting anti-NHba and/or anti-FHbp antibody in post-immunization sera on the percentage loss of SBA, we subtracted the respective pre SBA titer from both mock and antibody-depleted titers. The mean decrease in SBA titer after antibody depletion was compared to a theoretical decrease of 0% by a one sample t-test. The mean $\pm$ standard error of percent decreases from multiple assays was calculated using Prism GraphPad version 7.0 (GraphPad Software Inc, La Jolla, CA, USA. www.graphpad.com). For calculation of geometric mean titers, titers were log₁₀-transformed and titers below the lower limit of detection were assigned a value of one-half of the minimum detected titer (i.e., 1:2 for a titer <1:4).

Results

Adequacy of antibody depletion

We measured the serum IgG antibody reactivity before and after antibody depletion of post-immunization serum by ELISA. After the “mock-depletion”, there was an average of 29 to 30% decrease in the anti-FHbp or anti-NHba antibody titer compared to the respective original post-immunization serum (data not shown). This decrease represents serum dilution and/or loss of specific antibody.

Figure 1 shows the effect of depletion of serum anti-FHbp and/or anti-NHba antibodies on IgG antibody reactivity to the specific antigens. The results are expressed as ratios of the respective OD₄₀₅ values obtained with the antigen-depleted serum to mock-depleted serum (see methods). A ratio of 1 indicates that 100% of the antibody reactivity was retained and a ratio of 0.1 indicates that ~10% of the antibody was retained. For the 9 subjects, the anti-NHba antibody depletion decreased anti-NHba reactivity by a mean of 95% (ratio of NHba-depleted over mock-depleted sera of 0.05, Figure 1, **Panel A**). In contrast, there was no significant decrease in anti-FHbp reactivity after anti-NHba antibody depletion (ratio=0.99). Depletion of serum anti-FHbp antibody removed 94% of the anti-FHbp reactivity (ratio = 0.06) without decreasing anti-NHba reactivity (ratio=1.04, **Panel B**). Depletion of both anti-FHbp and anti-NHba antibody removed 95% of each of the respective antibodies but had no significant effect on depletion of antibodies to three control antigens, NadA, dOMV and tetanus toxoid (**Panel C**). The effect of depletion of anti-NHba or anti-FHbp antibody individually on antibody reactivity to the three control antigens was not measured since we inferred that if there was no evidence of non-specific loss of antibodies during double antibody depletion there also would be no loss after depletion of one antibody.

Effect of antibody depletion on serum bactericidal activity at 1-month post dose 2 or 3

All 9 immunized subjects showed large increases in SBA titer against strain 5/99 (Figure 2, **Panel A**), which expresses NadA but is mismatched for the other three MenB-4C antigens (Table 1). There were no significant changes in the post-immunization titers after depletion of both anti-FHbp and anti-NHba antibody. The effect of depletion of anti-NHba or anti-FHbp antibody individually on SBA titer to strain 5/99 was not measured since we inferred that if there was no evidence of non-specific loss of SBA after double antibody depletion there would be no loss after depletion of one antibody.

Seven subjects had >3-fold increases in SBA titer against strain SK016, which expresses PorA P1.4 but is mismatched for the other three MenB-4C antigens. Although this strain expresses sub-family A FHbp, subject 2 showed a 50% decrease in titer after depletion of anti-FHbp antibody (Figure 2, **Panel C**), and subject 8 showed a 50% decrease in titer after depletion of both anti-NHba and anti-FHbp antibodies. The remaining 5 subjects had <50% decreases in titer after depletion of both antibodies. However, for the group of seven responders, the mean decrease in SBA titer after depletion of anti-NHba antibody was 16% ($P=0.06$, compared to a theoretical decrease of 0% by a one sample t-test), 23% ($P=0.04$) after depletion of anti-FHbp antibody, and 36% ($P=0.003$) after depletion of both antibodies (Figure 2, **Panel D**). Thus, despite the strain antigens being matched for the vaccine only for PorA P1.4, antibodies to FHbp and, possibly, NHba, contributed to SBA responses.

Strain M4407 expresses NHba with 100% amino acid identity to the vaccine antigen and is mismatched for the other three MenB-4C antigens (Table 1). Depletion of serum anti-NHba antibodies decreased SBA by 50% in 6 of the 9 subjects. Data from representative subjects 4 and 8 with >50% decreases in SBA titer after depletion of anti-NHba antibody are shown in Figure 3, **Panel A**. One subject (Subject 2) was an outlier in that for reasons that are unknown, depletion of serum anti-NHba antibody increased SBA activity by >100% (data replicated in 3 different assays, Figure 3, **Panels A and B**). The increased SBA titer of subject 2 after depletion of anti-NHba antibodies was not observed against the other test strains in the panel (see for example, data for strain B2, below).

For the 8 subjects (excluding subject 2) with decreased SBA titers against strain M4407 after depletion of anti-NHba antibodies, the mean decrease was 63% compared to mock-depleted serum ($P<0.0001$). Although strain M4407 expresses FHbp sub-family A, depletion of serum anti-FHbp antibody also decreased SBA titers by 50% in 7 of the 9 subjects (see for example, data for subjects 2, 4 and 8 in **Panel A**). For all 9 subjects, the mean decrease in SBA titer after anti-FHbp depletion was 64%, and after depletion of both antibodies was 96% ($P<0.0001$, compared to a theoretical decrease of 0% by a one sample t-test for each antibody, **Panel B**).

In three post-immunization sera, depletion of both anti-NHba and anti-FHbp antibodies decreased SBA but the residual activity was above the respective pre-vaccination titers (see for example subject 8 in **panel A**). Since strain M4407 has PorA (P1.19,) and lacks a gene for NadA, the residual SBA after depletion of both anti-NHba and anti-FHbp antibodies suggests a bactericidal role for vaccine-induced antibodies to OMV antigens other than P1.4. In previous studies, adults immunized with OMV vaccines showed SBA responses to strains

with heterologous PorA VR sequence types to the vaccines [6]. The responsible OMV antigens were not defined but may include OpA (referred to as class 5 protein)[23, 24] and LOS [25].

We next tested SBA titers of antibody-depleted serum against two other strains, B2 and B9, which were matched with the vaccine antigens only for NHba (Table 1). For strain B2, depletion of serum anti-NHba antibodies decreased SBA by 50% in 3 of the 7 responders tested, with a mean decrease in all 7 subjects of 43% compared to mock depletion (Figure 4, **Panel A**, $P<0.03$, compared to a theoretical decrease of 0% by a one sample t-test). For strain B9, depletion of serum anti-NHba antibodies decreased SBA by 50% in all 6 responders tested with a mean decrease of 79% compared to mock depletion ($P<0.0001$, Figure 4, **Panel B**). The relatively greater effect of depletion of anti-NHba antibody on lowering SBA against strain B9 than strain B2 may be explained by higher NHba expression by strain B9 (Table 1). The increased SBA titer of subject 2 measured against strain M4407 after depletion of anti-NHba antibodies was not observed against strain B2 (depletion of serum anti-NHba antibodies decreased SBA by 60%, Figure 4, **Panel A**). The effect of depleting anti-NHba antibody on SBA of subject 2 against strain B9 could not be tested because of a <3 -fold SBA response of this subject to strain B9.

For Strain B2, depletion of anti-FHbp antibodies decreased SBA titers by 50% in 4 of the 7 subjects with a mean decrease of 49% in all 7 subjects ($P<0.0042$, compared to mock-depletion). Depletion of both antibodies decreased SBA by a mean of 84% ($P<0.0001$). For strain B9, depletion of anti-FHbp antibodies decreased SBA titers by 50% in two of the 6 subjects with a mean decrease in all six responders of 45% ($P<0.0023$ compared to mock adsorption). Depletion of both antibodies decreased SBA by a mean of 100% ($p<0.0001$). Thus, with the exception of subject 2 against strain M4407, the data for the three strains matched with the vaccine only for NHba indicated that antibodies to NHba individually, or FHbp individually, can contribute to vaccine-induced SBA while antibodies to both antigens together had the most activity.

For the Princeton University strain, which expresses both FHbp sub-family B and NHba that match the vaccine antigens (Table 1) there were no subjects in whom depletion of serum anti-NHba antibodies alone decreased SBA by 50% whereas depletion of anti-FHbp alone decreased SBA titers by 50% in all 9 subjects. In 9 subjects, depletion of anti-NHba antibody decreased SBA by a mean of 26% compared to mock-depletion ($P<0.0001$, compared to a theoretic mean decrease of 0% by a one sample t-test, Figure 5, **Panel A**). Depletion of anti-FHbp antibody decreased SBA by a mean of 92% ($P<0.0001$). Thus, despite relatively high strain expression of NHba with 100% amino acid identity to the vaccine antigen, anti-NHba antibodies in the absence of anti-FHbp antibody had negligible SBA against this strain.

For the Rutgers University outbreak strain, which is mismatched for all four antigens in MenB-4C (Table 1), 6 subjects had >3 -fold increases in SBA titer. Depletion of serum anti-NHba antibody decreased SBA by a mean of 48% ($P<0.003$) while depletion of anti-FHbp antibody decreased SBA by a mean of 100% (Figure 5, **Panel B**). Thus, despite the mismatched FHbp sub-family antigen in this strain, SBA required anti-FHbp antibody.

While there was sufficient NHba expression by the Rutgers University strain for anti-NHba antibodies to augment anti-FHbp SBA titers, the residual serum anti-NHba antibodies after depletion of anti-FHbp antibodies had no detectable SBA.

Discussion

NHba (previously designated Genome-derived Neisserial Antigen 2132 (GNA2132) is a lipoprotein present in nearly all meningococcal strains [26]. While the function of NHba is unknown, the protein binds heparin, which may increase resistance of *N. meningitidis* to complement [27]. Early pre-clinical studies showed that mice immunized with recombinant NHba developed SBA responses mainly with rabbit complement [1, 15]. Two previous studies investigated the bactericidal role of anti-NHba antibodies in humans using human complement. In adults immunized with a vaccine containing the three recombinant proteins in MenB-4C (FHbp, NHba and NadA i.e., MenB-3C), antibodies to NHba alone lacked SBA against strain M4407 [18]. However, based on antibody depletion studies, vaccine-induced anti-NHba antibodies augmented SBA elicited by cross-reacting anti-FHbp sub-family B antibodies (interpreted as showing anti-NHba and anti-FHbp cooperativity [18]). The Vu study used antigens coupled to a solid matrix to deplete specific serum antibody. In contrast, a study by Giuliani et al added soluble FHbp or NHba to the bactericidal reaction to inhibit antibody reactivity [28]. They found that the addition of NHba removed complement-mediated SBA against M4407 while the addition of FHbp did not decrease SBA. The results are difficult to interpret since the addition of FHbp to the bactericidal reaction mixture would be expected to remove both anti-FHbp and complement FH. In the absence of FH (a complement down-regulator) [29, 30] the strain would have increased susceptibility to anti-NHba SBA.

In the present study, we used recombinant antigens coupled to a solid phase to deplete serum antibody to investigate the bactericidal role of anti-NHba antibody induced in adults by MenB-4C vaccination. Our most important finding was that anti-NHba antibody contributed to SBA against the three meningococcal B strains that expressed NHba and were mismatched for the other three MenB-4C antigens. However, as previously seen in the MenB-3C vaccine study [18], cross-reactive antibodies to FHbp sub-family A also contributed to SBA, either alone or in concert with anti-NHba antibodies. Similar results were observed against a fourth strain from an outbreak at Rutgers University, which was considered mismatched for all four MenB-4C antigens. Against a fifth test strain from an outbreak at Princeton University, which was matched with the vaccine for both FHbp sub-family B and NHba, nearly all of the SBA was dependent on anti-FHbp antibodies despite strain expression of NHba with 100% amino acid identity with the vaccine antigen. These data indicate that in the absence of anti-FHbp antibodies, some strains can be resistant to anti-NHba bactericidal activity despite NHba expression [15].

Four of the 9 subjects (3, 4, 5 and 8) in our study had >3-fold SBA responses against all five test strains used to characterize the role of anti-NHba or anti-FHbp antibody in SBA. Figure 6 shows the percent loss of SBA after antibody depletion for each of these subjects and for the five test strains. For all four subjects, depletion of both anti-NHba and anti-FHbp antibodies removed all, or nearly all, of the SBA (black bars). However, the effect of

depletion of an individual antibody varied depending on the strain, individual subject, and antibody specificity. For example, the principal bactericidal antibody for the Princeton and Rutgers University strains was anti-FHbp (checkered bars), whereas for strain B9, it was anti-NHba (striped bars). For all four subjects, depletion of anti-FHbp antibodies elicited by the sub-family B vaccine antigen contributed by varying amounts to SBA against the four test strains with FHbp subfamily A (M4407, B9, B2 and Rutgers University).

For our studies, we required sufficient SBA responses to strain M4407 with high NHba expression, to determine the effect of specific antibody depletion on SBA. Our studies were therefore limited to nine subjects with >3-fold SBA responses (five given 2 doses and 4 given 3 doses). While we were unable to determine the bactericidal role, if any, of anti-NHba antibody in sera from immunized persons with lower SBA responses, our data are likely representative of the contribution of anti-NHba antibody on SBA responses of immunized adults with >3-fold SBA responses.

In summary, anti-NHba antibody can contribute to SBA elicited by MenB-4C, particularly in concert with anti-FHbp antibody. However, only 40 percent of 20 adults vaccinated with two doses had >3-fold SBA responses against strains with moderately high NHba expression and, in those who responded, a large proportion of the SBA was directed at FHbp sub-family A antigens. Thus, the mechanisms by which the NHba antigen in MenB-4C elicits SBA are complex and will vary depending on antibody composition of an individual's serum, antigenic composition of the strain, and possibly other intrinsic strain factors that can affect complement regulation such as binding of Factor H to NspA or PorB [31]. The seemingly complex nature of these interactions raises questions about the use of NHba expression measured by the meningococcal antigen typing system (MATS) [32] for predicting strain coverage, particularly for strains "matched" with the vaccine only for NHba.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

1. MenB-4C (Bexsero®) is a multicomponent serogroup B meningococcal vaccine.
2. The bactericidal role of antibody to one of the four antigens, Neisserial Heparin binding antigen (NHba), is incompletely understood.
3. In 9 immunized adults, anti-NHba antibody contributed to SBA against some strains, particularly in concert with anti-FHbp antibody.
4. One high NHba-expressing strain with an amino acid sequence identical to the vaccine was resistant.
5. Predicting anti-NHba strain coverage is challenging based on antigen expression and reactivity

Figure 1. Adequacy and specificity of antibody depletion as measured by ELISA in post-immunization sera

Antibody depletion expressed as a ratio of OD values when testing reactivity of paired antigen-depleted or mock-depleted sera at a dilution of 1:100. For a few sera with similar reactivity of the mock-depleted serum at dilutions of 1:100, 1:200 or 1:400, we used the respective OD values of the mock- and antigen-depleted serum at the highest dilution on the plateau **A**, Anti-NHba, **B**, Anti-FHbp, and **C**, Anti-FHbp + anti-NHba. Antibody reactivity was measured against NHba, FHba and three control antigens, NadA, detergent-treated outer membrane vesicles (dOMV from strain NZ98/254) and tetanus toxoid (TT). The effect of depletion of anti-NHba or anti-FHbp antibody individually on antibody reactivity to the three control antigens was not measured since we inferred that if there was no evidence of non-specific loss of antibodies during double antibody depletion there also would be no loss after depletion of an individual antibody.

Figure 2. Effect of depletion of serum anti-FHbp and anti-NHba antibody on serum bactericidal activity measured against control strains 5/99 (NadA) and SK016 (PorA P1.4)

A and C, Titters measured against strains 5/99 and SK016, respectively. For 5/99, titers of the 9 subjects were measured in one assay. For SK016, data are mean values for each subject from four replicate assays. Subjects 1 and 7 were non-responders (NR) with <3-fold increases in titer against strain SK016 and, therefore, were not measured against this strain. **B and D.** Mean percent change in titer after antibody depletion of post-immunization serum against strain 5/99 and SK016, respectively, compared with respective mock-depleted sera. For strain SK016 (Panel D), the decreases in SBA titer after depletion of anti-FHbp antibody or depletion of both antibodies were significant, compared to a theoretical decrease of 0% (* $P=0.04$; and ** $P=0.003$, respectively).

Figure 3. Effect of depletion of anti-FHbp and/or anti-NHba on serum bactericidal activity against strain M4407

M4407 has moderately high expression of NHba with 100% amino acid identity to NHba in MenB-4C [18] and is mismatched for the other three MenB-4C antigens. All 9 subjects had >3-fold increase in titer at 1-month post-vaccination compared to pre-titers. **A.** Titers (mean $\pm$ SE from 3 assays) in mock- and FHbp- or NHba-adsorbed sera for representative subjects 4, 8 and 2. Subjects 4 and 8 showed >50% decreases in titer after anti-NHba depletion. Subject 2 showed an increase ($P=0.025$) in titer in post-immunization sera after depletion of anti-NHba antibody compared to mock-depletion. **B.** Mean percent change in titer after antibody depletion of post-immunization sera from 8 subjects compared to mock-adsorbed (subject 2 excluded) and mean percent change in titer after antibody depletion of post-immunization serum from subject 2.

Figure 4. Effect of depletion of anti-FHbp and/or anti-NHba on serum bactericidal activity against strains B2 and B9

Mean percent change in titer after antibody depletion compared to mock-depletion for seven subjects tested with >3-fold increases in titer against strain B2 (**panel A**), and for six subjects with >3-fold increases in titer against strain B9 (**panel B**). Data are from 4 replicate assays. Strain B2 has + expression of NHba with 88% amino acid identity to vaccine, and strain B9 has ++ expression with 87% amino acid identity (Table 1). Both strains are mismatched for the other three MenB-4C antigens (Table 1).

Figure 5. Effect of serum antibody depletion on bactericidal activity against two college outbreak strains

Mean percent decrease in titer for subjects with >3-fold responses after antibody depletion compared to mock-depletion. **A**, Nine responders to the Princeton University strain, which is matched with MenB-4C for FHbp sub-family and NHba. **Panel B**, Six responders to the Rutgers University strain, which is mismatched for all four MenB-4C antigens (Table 1). Data from individual subjects are from 3 or 4 replicate assays.

Figure 6. Effect of serum antibody depletion on bactericidal activity for four subjects with >3-fold responses against all five test strains

Data are from 3 to 4 replicate assays for subjects 3, 4, 5, and 8. Strains M4407, B2 and B9 are matched with the vaccine for NHba with high to moderate expression. Princeton University strain is matched with the vaccine for NHba and FHbp sub-family B, while Rutgers University strain is mismatched for all four vaccine antigens. The antigens shown in brackets represent those “matched” to antigens in MenB-4C (See Method section).

Table 1

Meningococcal strains used for testing serum bactericidal activity

Strain (reference)	Alternative Designation(s)	No. Sera Tested ^a	Location (Year)	MLST (clonal complex) ^b	PorA Serosubtype	FHbp Sub-family (Peptide ID) ^c	NHba Peptide ID ^c	Strain Antigen Expression ^d			Antigen(s) matched with vaccine
								FHbp	NHba	NadA	
5/99 [4, 33]		9	Norway (1976)	1349 (8)	P1.2	A (23)	20	+	+/-	++	NadA
SK016 [5, 19]	NM01593	7	North Carolina (2001)	103 (103)	P1.4	A (25)	ND	+	+/-	-	PorA P1.4
M4407 [5, 18]	B11	9	Minnesota (1996)	6160 (41/44)	P19, 15-1	A (19)	2	++	++	-	NHba
B2 [5]	CH740, M11051	7	Georgia (2003)	13 (269)	P19, 15	A (19)	6	+	+	-	NHba
B9 [5]	CH687, M03279	6	Georgia (1997)	136 (41/44)	P17, 16-3	A (24)	11	++	++	-	NHba
Rutgers Univ. [20]	CH865, M39811	6	Rutgers Univ (2016)	11 (11)	P1.5-1, 10-1	A (19)	20	+/-	+/-	-	None
Princeton Univ. [15, 34]	CH819, M26312	9	Princeton Univ (2013)	409 (41/44)	P5-1, 2-2	B (276)	2	+	+	-	FHbp & NHba

^aNine subjects were selected with >3-fold SBA responses to strain M4407 (See Supplemental Tables S1 and S2, and methods). Shown are the number of sera from these 9 subjects with >3-fold responses to the other six strains (ranging from 6 to 9 sera for each strain)

^bMLST, multilocus sequence typing [35]

^cFHbp and NHba peptide ID as described in the public databases (<https://pubmlst.org/neisseria/FHbp/>) and <https://pubmlst.org/neisseria/NHBA/>)

^dAntigen expression was measured by flow cytometry (see methods): +/-, <20% expression compared to a positive control strain tested in parallel; +, 20–100% expression; ++, 100% expression. All strains except 5/99 lacked a gene for NadA.