Complement-Dependent Synergistic Bactericidal Activity of Antibodies against Factor H–Binding Protein, a Sparsely Distributed Meningococcal Vaccine Antigen

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Background. Antibodies to factor H (fH)–binding protein (fHBP), a meningococcal vaccine antigen, activate classical complement pathway serum bactericidal activity (SBA) and block binding of the complement inhibitor fH.

Methods. To understand these 2 functions in protection, we investigated the interactions of human complement and 2 anti-fHBP monoclonal antibodies (MAbs) with encapsulated Neisseria meningitidis.

Results. JAR 3 (IgG3) blocks fH binding and elicits SBA against 2 strains with naturally high fHBP expression and a low-expressing strain genetically engineered to express high fHBP levels. JAR 4 (IgG2a) does not block fH binding or elicit SBA. Neither MAb alone elicits SBA against 2 other strains with low fHBP expression, but together the MAbs increase C4b binding and elicit SBA; JAR 3 alone also is bactericidal in whole blood. In nonimmune blood, fHBP knockout mutants from high-expressing stains do not survive, but mutants of low-expressing strains do.

Conclusions. Expression of fHBP is a prerequisite for bacterial survival in blood only by strains with naturally high fHBP expression. In low-expressing strains, combinations of 2 nonbactericidal anti-fHBP MAbs can bind to nonoverlapping epitopes, engage C1q, activate C4, and mediate classical complement pathway SBA. In the absence of sufficient C4b binding for SBA, an individual MAb can have opsonophagocytic bactericidal activity.

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strains with relatively high expression of fHBP but were not bacterial against other strains with lower expression of fHBP. When 2 nonbactericidal MAbs were combined, some of the combinations had bactericidal activity against resistant (low fHBP-expressing) strains. The purposes of the present study were to investigate the basis for this bactericidal synergy and to examine survival in human blood of N. meningitidis strains that naturally produce low or high amounts of fHBP and their respective fHBP knockout mutants.

METHODS

Recognition of overlapping or nonoverlapping fHBP epitopes by JAR 3 and JAR 4. The ability of JAR 3 (IgG3) to inhibit binding of JAR 4 (IgG2a) or of JAR 4 to inhibit binding of JAR 3 to solid-phase variant 1 recombinant fHBP was performed by ELISA, as described elsewhere [8]. The secondary antibodies for detection of JAR 3 or JAR 4 were alkaline phosphatase−conjugated goat anti-mouse antiserum specific for IgG subclasses 3 or 2a, respectively (Jackson ImmunoResearch).

Bacterial strains. Three of the N. meningitidis strains were capsular group B; 2 (H44/76 and MC58) were sequence types (STs) 32 and 74, respectively (both from ST 32 complex [9−11]); and 1 (NZ98/254) was representative of ST 41/44 complex [12]. The fourth strain, 4243 [13], was group C and ST 11. Our approach to increasing the expression of fHBP from strain NZ98/254 was similar to that described elsewhere [14, 15], except that we used a different plasmid (pComPind; gift from I. Delany, Novartis Vaccines) that integrates into the chromosome and allows expression of fHBP under control of the strong promoter from nmb1523 [16]. Strain NZ98/254, in which the gene encoding fHBP had been inactivated as described elsewhere [14], was transformed with pComP1523 containing the full-length gene of fHBP from strain NZ98/254. The transformation was performed as described elsewhere [15], and transformants were selected on GC agar plates containing 5 µg/mL chloramphenicol. To measure fHBP expression in the mutants, we isolated outer membrane vesicles released by bacteria into the medium, as described elsewhere [17], and used Western blot analysis [15] with anti-fHBP variant 1 MAb, JAR 3.

Complement-dependent bactericidal antibody activity. The bactericidal assay was performed as described elsewhere, using bacteria grown for approximately 2 h (to an absorbance at 620 nm of ~0.6) in Mueller-Hinton broth supplemented with 0.25% glucose (wt/vol) and 0.02 mmol/L cytidine 5′-monophospho-N-acetylneuraminic acid (Sigma-Aldrich) [2, 18]. We used Gey’s balanced salt solution containing 1% bovine serum albumin (A-2153; Sigma-Aldrich) to wash and resuspend the bacteria and to dilute the MAb. The complement source was serum from a nonimmune healthy adult whose serum had normal hemolytic complement activity and had no detectable intrinsic bactericidal activity when tested at 40% (twice the concentration used in the assay to test activity of the MAbs).

Survival of N. meningitidis in human blood or plasma. Details of the whole blood and plasma bacterial survival assay have been described elsewhere [19]. Informed consent was obtained from both donors, and the guidelines of the institutional review board were adhered to, using an approved protocol. To avoid the effects of heparin or chelation on complement activation, we used recombinant hirudin, a specific thrombin inhibitor, as the anticoagulant (lepirudin [rDNA; Refludan, Berlex] [20]).

Detection by flow cytometry of indirect fluorescence on live bacterial cells. Binding of anti-fHBP MAbs to the bacterial surface was measured by flow cytometry using live encapsulated meningococci, as described elsewhere [2, 21, 22]. The anti-fHBP antibody-dependent deposition of human complement component C4b was measured using polyclonal anti-C4−fluorescein isothiocyanate (FITC; Biodesign), as described elsewhere, except that Gey’s buffer was used instead of Hank’s balanced salt solution with calcium and magnesium [6]. Binding of fH was measured using purified human fH (Complement Technology). The protein (10 µg/mL) was added to suspensions of bacteria, which were prepared as described for the bactericidal assay, and the suspension was incubated at 37°C for 20 min. After washing, the cells were incubated with a 1:100 dilution of goat anti-human fH polyclonal antibody (Bethyl Laboratories). Bound fH was detected using FITC-conjugated anti–goat IgG (Sigma-Aldrich), as described elsewhere [6].

RESULTS

Recognition of nonoverlapping epitopes by anti-fHBP MAbs JAR 3 and JAR 4. To investigate whether the MAbs JAR 3 and 4 recognize overlapping or nonoverlapping epitopes, we used an ELISA to measure the ability of each MAb to bind to recombinant fHBP when the second MAb was present in excess (50 µg/mL). Binding of JAR 3 (0.05 µg/mL) and JAR 4 (0.06 µg/mL) was detected with anti-IgG3 or anti-IgG2a mouse polyclonal antiserum, respectively, each separately conjugated to alkaline phosphatase.

Binding of JAR 3 was not inhibited by JAR 4 (figure 1A), and binding of JAR 4 was not significantly inhibited by JAR 3 (figure 1B). The positive control, a 1:10 dilution of rabbit anti-fHBP antisera, inhibited binding of JAR 3 and JAR 4 by 64% and 95%, respectively, compared with <7% for preimmune rabbit serum and negative control MAbs. Together with our published findings that only JAR 3 inhibits binding of fH to N. meningitidis.
[6], these data indicate that JAR 3 and 4 recognized nonoverlapping epitopes.

**Bactericidal activity of anti-fHBP MAbs.** JAR 3 was bactericidal against strain H44/76 (50% killing of the bacteria [BC$_{50}$] occurred at 0.3 µg/mL) (figure 2), whereas JAR 4 was not bactericidal (BC$_{50}$ > 100 µg/mL). When the 2 MAbs were combined, the bactericidal activity of the combination was similar to that of JAR 3 alone. Similar respective bactericidal activities for each of the MAbs individually or in combination were observed with a second strain, MC58 (figure 2), although bacterial survival decreased at the highest concentrations of JAR 4 tested. Neither JAR 3 nor JAR 4 individually was bactericidal (BC$_{50}$ > 100 µg/mL) against 2 other strains, NZ98/254 and 4243, but the combination was bactericidal at concentrations as low as 1 µg/mL against strain NZ98/254 and 2 µg/mL against strain 4243 (figure 2 and table 1).

**Strain-dependent expression of fHBP.** One possible explanation for the susceptibility of some but not all strains to complement-mediated bactericidal activity of JAR 3 is that the susceptible strains express more fHBP than the resistant strains. To investigate this possibility, we used flow cytometry to measure dose-response binding of anti-fHBP antibody to the surface of live *N. meningitidis* cells (figure 3A). Cells from strains H44/76 (susceptible to JAR 3 alone) and NZ98/254 (resistant to JAR 3) showed similar respective dose-response binding with a control anticapsular MAb, with the highest fluorescence intensity at a 50 µg/mL concentration of MAb (figure 3A, black areas of histograms), intermediate binding at 2 µg/mL (white areas), and minimal binding at the lowest concentration tested, 0.4 µg/mL (dark gray areas). With strain H44/76, the binding of JAR 3 at 50 µg/mL was similar to that at 2 µg/mL (black and white areas are superimposed), but binding was decreased at 0.4 µg/mL. With strain NZ98/254, all 3 concentrations of JAR 3 gave the same intensity of fluorescence. Thus, there was saturation of binding at a JAR 3 concentration of 2 µg/mL with strain H44/76, compared with 0.4 µg/mL for strain NZ98/254. There was similar saturation of binding at 2 µg/mL for JAR 3 with strain MC58 (susceptible to JAR 3 bactericidal activity), compared with 0.4 µg/mL for saturation with the JAR 3-resistant strain, 4243 (data not shown). These results are consistent with lower expression of fHBP in strains NZ98/254 and 4243 than in strains H44/76 and MC58.

We also used flow cytometry to measure the binding of fH to live bacterial cells of the different strains. There was 10-fold more fH binding fluorescence with strain H44/76 than with NZ98/254 (figure 3B). Similar higher binding of fH was observed with strain MC58 compared with strain 4243 (data not shown).

The results described above were consistent with the hypothesis that resistance of strains 4243 and NZ98/254 to complement-mediated bactericidal activity of JAR 3 arises from insufficient antibody deposition on the bacterial surface because of relatively low expression of fHBP by these strains. To test this possibility directly, we engineered a mutant of strain NZ98/254 with increased expression of fHBP (figure 4A) and investigated the ability of JAR 3 to mediate complement-dependent killing (figure 4B). With the mutant, the MAb alone was bactericidal. The bactericidal activity was specific for JAR 3, because JAR 4 was not bactericidal. Also, the mutant did not have increased susceptibility to bactericidal activity of a positive control anti-P1.4 PorA MAb (titers against the mutant and wild-type strains, 1:50 and 1:250, respectively). Thus, the bactericidal activity of JAR 3 individually against the mutant with increased fHBP ex-

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*Figure 2.* Bactericidal activity by monoclonal antibodies (MAbs) to factor H–binding protein (fHBP), individually or in combination. MAbs include JAR 3 (black squares), JAR 4 (white circles), JAR 3 plus JAR 4 (asterisks, dashed lines), and anti-PorA MAb as a positive control (black circles).
pression paralleled the observations that JAR 3 individually was bactericidal only against the 2 naturally high fHBP-expressing strains, H44/76 and MC58 (figure 2).

**C1q engagement and activation of the classical complement pathway.** The classical pathway of complement activation on a surface is initiated when a sufficient density of antigen-antibody complexes allows proximate Fc regions of the antibody to bind C1q present in the C1 complex. C1 engagement in turn activates C4, which ultimately leads to C3b deposition, membrane attack complex formation, and bacteriolysis. We therefore used indirect flow cytometry to measure C4b binding to the surface of live *N. meningitidis* cells as a surrogate marker for C1q binding and C4 activation.

When anti-PorA MAbs and 20% nonimmune human serum as a complement source were incubated with live bacteria of strains NZ98/254 or H44/76, C4 was activated with subsequent deposition of C4b on both strains (figure 5, row 1, white areas of histograms). This activity was sufficient to elicit bactericidal activity (figure 2). With JAR 4 individually, C4b deposition on either strain was negligible (row 3), whereas with JAR 3 (row 2) there was C4b deposition on both strains, but the fluorescence intensity was ~10-fold higher for the higher fHBP-expressing strain, H44/76, than for the lower fHBP-expressing strain, NZ98/254.

When both strains were incubated with a combination of JAR 3 and JAR 4 (row 4), C4b deposition on the higher fHBP-expressing strain, H44/76, was similar to that observed with JAR 3 alone, whereas C4b deposition on the lower-expressing strain, NZ98/254, increased ~5-fold with the combination of MAbs, compared with JAR 3 alone. The C4b binding by the combination was sufficient to elicit bactericidal activity (figure 2). Although not shown in figure 5, the respective C4b results with the second fHBP low-expressing strain, 4243, paralleled those observed with the low-expressing strain NZ98/254. Thus, with both low-expressing strains the combination of the 2 MAbs, but not the individual MAbs, activated the classical complement pathway sufficiently for bactericidal activity.

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**Table 1. Bactericidal activity of antibodies to factor H–binding protein (fHBP) against *Neisseria meningitidis.***

<table>
<thead>
<tr>
<th>Antibody</th>
<th>BC50, µg/mL</th>
<th>High fHBP-expressing strains</th>
<th>Low fHBP-expressing strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H44/76 (B:15; P1.7, 16)</td>
<td>MC58 (B:15; P1.7, 16-2)</td>
</tr>
<tr>
<td>Anti-fHBP MAb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAR 3 (IgG3)</td>
<td>0.3</td>
<td>2.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td>jAR 4 (IgG2a)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>JAR 3+4</td>
<td>0.2</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Anticapsular MAb</td>
<td>2.0</td>
<td>15</td>
<td>1.0</td>
</tr>
<tr>
<td>Anti-PorA MAb</td>
<td>0.2</td>
<td>2.0</td>
<td>0.2</td>
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<td>Anti-capsular MAb</td>
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**NOTE.** BC50 is the concentration of monoclonal antibody (MAb) that yields a 50% decrease in colony-forming units per milliliter after a 1-h incubation with bacteria and human complement, compared with controls at time 0. Data shown are the geometric mean concentrations for at least 3 separate experiments. No killing was observed with complement in the absence of the MAb or with complement together with an irrelevant MAb.

- Capsular group, PorB serotype, and PorA variable region sequence type (respectively) are given in parentheses.
- Group B anti-capsular MAb SEAM 12 [22] for strains H44/76, MC58, and NZ98/254 and group C anti-capsular MAb JW-C2 for strain 4243; see Methods.
- Anti-P1.7 for strain H44/76 and MC58, anti-P1.2 for strain 4243, and anti-P1.4 for strain NZ98/254, on the basis of the specific PorA P1 epitope expressed by each of the strains.
Expression of fHBP and survival of N. meningitidis in nonimmune human blood. We measured the survival in nonimmune human blood of 2 naturally high fHBP-expressing strains, H44/76 and MC58, and their respective isogenic mutants in which the genes encoding fHBP had been inactivated (H44/76 ΔfHBP and MC58 ΔfHBP). The results were compared with survival of the 2 naturally low fHBP-expressing strains, NZ98/254 and 4243, and their respective isogenic fHBP knockout mutants (NZ98/254 ΔfHBP and 4243 ΔfHBP).

After incubation at 37°C for 3 h in the blood of 2 healthy nonimmune adults, the number of colony-forming units per milliliter increased for all 4 wild-type strains (figure 6A). In contrast, within 1–2 h, the 2 fHBP knockout mutants from the naturally high-expressing strains did not survive, whereas the 2 fHBP knockout mutants from the low fHBP-expressing strains did survive. Failure of the H44/76 ΔfHBP and MC58 ΔfHBP mutants to survive in blood did not result from an intrinsic defect in growth, because both mutants showed similar growth curves compared with their respective wild-type strains in Mueller-Hinton broth (figure 6B). To demonstrate that poor survival of the H44/76 ΔfHBP mutant in nonimmune blood was caused by lack of expression of fHBP, we measured survival of a second mutant of H44/76 ΔfHBP that had been transformed with a plasmid encoding fHBP, which enhanced fHBP expression [15]. As shown in figure 6C, the knockout mutant transformed with the plasmid and expressing fHBP showed growth in blood similar to that of the wild-type strain, whereas the H44/76 ΔfHBP mutant without the plasmid did not survive. Together, these data indicate that survival of the 2 naturally high fHBP-producing strains in nonimmune human blood was dependent on expression of fHBP, whereas survival of the 2 naturally low fHBP-expressing strains did not require fHBP expression.

Opsonophagocytic bactericidal activity of anti-fHBP MAb.

Neither JAR 3 nor JAR 4 alone was bactericidal against the 2 low fHBP-producing strains, 4243 or NZ98/254, in a bactericidal assay using 20% nonimmune human serum as the com-

Figure 4. Susceptibility to JAR 3 bactericidal activity of a mutant of NZ98/254 engineered to increase factor H–binding protein (fHBP) expression. A, Expression of fHBP in outer membrane vesicles (OMVs), as measured by Western blot analysis with JAR 3. Wild type, OMV from parent NZ98/254 strain; KO, OMV from knockout strain with inactivation of the gene encoding fHBP; mutant, OMV from mutant engineered to have increased expression of fHBP. B, Bactericidal activity by anti-fHBP monoclonal antibody (MAb) JAR 3 against a mutant of NZ98/254 engineered to increase expression of fHBP. JAR 3 was tested against a wild-type strain (black squares, dashed line) or a mutant with increased expression of fHBP (white squares, solid line), and JAR 4 was tested against a wild-type strain (black circles, dashed line) or a mutant (white circles, solid line).

Figure 5. Binding of human C4b to the surface of live encapsulated Neisseria meningitidis cells, as determined by indirect fluorescence flow cytometry. The left column shows results for the group B strain H44/76, and the right column shows results for the group B strain NZ98/254. Black areas in histograms indicate bacteria with 5% nonimmune human serum, and white areas indicate bacteria with monoclonal antibodies and 5% nonimmune human serum. Row 1: left, anti-PorA, P1.7, 2 μg/mL; right, anti-PorA, P1.4, 10 μg/mL. Row 2: JAR 3, 10 μg/mL. Row 3: JAR 4, 50 μg/mL. Row 4: JAR 3 plus JAR 4 (1 μg/mL for each).
mercial source (figure 2). However, because JAR 3 alone activated some C4b deposition (figure 5), it was possible that this MAb could have bactericidal activity in whole blood, mediated by the higher concentrations of complement and/or opsonophagocytosis. In a whole blood assay (figure 7), JAR 3 was bactericidal against both low fHBP-expressing strains. The activity was largely dependent on the presence of white cells, because when JAR 3 was tested in plasma, there was <0.5 log10 decrease in colony-forming units per milliliter during the 60 min of incubation. JAR 4 alone was not bactericidal in whole blood or plasma against either of the 2 strains (BC50 > 50 μg/mL).

**DISCUSSION**

Binding of the complement protein fH is recognized as an important mechanism by which pathogens evade the innate system (reviewed in [23]). Bacterial pathogens reported to bind fH include *Streptococcus pyogenes* [24, 25], *Streptococcus agalactiae* [26], *Borrelia burgdorferi* [27–30], *Streptococcus pneumoniae* [31, 32], and *Neisseria gonorrhoeae* [33, 34]. With *N. meningitidis*, binding of fH enhances survival of the organism in normal human serum [6, 7]. Additional indirect evidence that fH contributes to the pathogenesis of meningococcal disease comes from a
recent report that persons homozygous for a single-nucleotide polymorphism, C-496T, have increased serum fH protein levels, which are associated with an increased risk of developing meningococcal disease [35].

fHBP is unique in that most vaccine antigens are abundant on the surface of a pathogen, whereas fHBP epitopes are sparsely distributed on most strains of *N. meningitidis* [1, 2]. Despite the sparseness, polyclonal anti-fHBP antibodies elicited complement-mediated bactericidal activity against genetically diverse strains [1, 4, 36] and inhibited binding of fH to the bacterium [6]. Inhibition of fH binding would be expected to increase susceptibility of the organism to complement-mediated killing.

Our data (figure 6) showing poor survival in human blood of fHBP-deletion mutants prepared from *N. meningitidis* strains with naturally high fHBP expression are consistent with the hypothesis that expression of fHBP is important for evasion of nonimmune host defenses by some strains. However, for strains that naturally produce lower amounts of fHBP, fH binding appears to be less important for survival, because fHBP knockout mutants from these strains survived in human blood. It is likely therefore that some strains with naturally low fHBP expression have developed alternative mechanisms for evading complement activation in the nonimmune host.

In a serum bactericidal assay using 20% nonimmune serum as a complement source, the control anti-PorA or anticapsular antibodies were bactericidal against all 4 wild-type strains (table 1). Yet the anti-fHBP MAb JAR 4 was not bactericidal against any of the strains, and JAR 3 was bactericidal only against the 2 strains with relatively high fHBP expression. The low-expressing strains required both MAbs together for complement-mediated bactericidal activity. However, JAR 3 alone had serum bactericidal activity when tested against a mutant of a low-expressing strain that had been engineered to increase expression of fHBP (figure 4).

JAR 3 and 4 bind to nonoverlapping epitopes because, as shown by ELISA, neither MAb inhibited binding of the other MAb to fHBP (figure 1). JAR 3 but not JAR 4 also inhibited binding of purified fH to *N. meningitidis* [6]. The spatial orientation and density of antigens on a surface of a bacterium can be important in determining whether “suitable pairs” of IgG directed against that antigen can support C1q binding and complement activation [37, 38]. In the case of fHBP, failure of an individual anti-fHBP MAb to activate complement-mediated bacteriolysis of a low fHBP-expressing strain could result if the distance between most of the fHBP molecules on the bacterial surface exceeded that required for optimal C1q engagement by antibody binding. Furthermore, blocking fH binding in the absence of optimal C1q engagement may not be sufficient to mediate bactericidal activity by complement, particularly in low fHBP-expressing strains. In contrast, binding of 2 antibodies to a low fHBP-expressing strain could be sufficient for adjacent Fc domains to engage C1q and activate bactericidal activity by the classical complement pathway, as long as the 2 antibodies recognized appropriately spaced nonoverlapping epitopes. The C4b deposition experiments (figure 5) reflect C1q engagement and the ability of the C1 complex to activate C4 [39]. The results were consistent with the hypothesis that binding of a single MAb is...
insufficient to engage adequate amounts of C1q for effective classical complement activation, whereas binding of 2 antibodies engages sufficient C1q if the antibodies recognize nonoverlapping epitopes.

With respect to expression of fHBP, N. meningitidis strains can broadly be subdivided into 2 groups on the basis of genetic lineages: ST 32 complex strains (e.g., H44/76 and MC58) express a highly conserved and relatively abundant protein [1, 2, 36], whereas strains from other clonal complexes, such as ST 11 (strain 4243) or ST 41/44 (strain NZ98/254), express proteins that can have polymorphisms affecting anti-fHBP binding [2, 36] and have less fHBP than ST 32 complex strains [1]. To date, all ST 32 complex strains tested have been highly susceptible to the bactericidal activity of individual anti-fHBP MAbs, such as JAR 1 [1], JAR 3 [2, 36], or MAb 502 [40]. In contrast, none of the 12 MAbs we have prepared that recognize fHBPs from 1 or more of each of the 3 major variant groups is individually bactericidal with human complement against non-ST 32 complex strains; yet, as described here for JAR 3 and 4, different combinations of each of the MAbs can be bactericidal against non-ST 32 complex strains. These data, together with the results presented above, underscore the correlation between low expression of fHBP by a strain and the requirement for >1 anti-fHBP MAb to elicit bactericidal activity.

Although JAR 3 individually did not elicit serum bactericidal activity against the 2 fHBP low-expressing strains, 4243 and NZ98/254, this MAb was bactericidal against these strains in the whole blood assay (figure 7). In contrast, JAR 4 individually was not bactericidal against these strains when tested in serum or whole blood. In our previous study, JAR 3 activated ~5-fold greater C3b deposition against these strains than did JAR 4 [2], which is consistent with the present data showing greater C4b activation by JAR 3 alone than by JAR 4 (figure 5).

In conclusion, our data provide evidence that a protein antigen that is sparsely present on the bacterial surface of most strains of encapsulated N. meningitidis can be an effective vaccine candidate, provided that the antibody responses are directed against >1 nonoverlapping epitope, permitting effective classical complement pathway activation. This situation would normally be the case with polyclonal antibodies elicited by a protein antigen. In the absence of serum bactericidal activity, our data also suggest that an individual anti-fHBP MAb can bind and activate sufficient complement deposition for whole blood opsonophagocytic bactericidal activity via Fc and iC3b receptors. Although the role played by opsonophagocytic bactericidal activity in protecting against meningococcal disease without serum bactericidal activity is controversial [41], support for a protective function comes from recent seroepidemiologic [42], immunization [43], and experimental studies [19, 43–45]. Finally, differences in natural levels of expression of fHBP are important determinants of the susceptibility of a strain to anti-fHBP bactericidal activity as well as the mechanism used by the organism to evade nonimmune host defenses.

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References

Figure 1. Recognition of nonoverlapping epitopes by JAR 3 and 4. A, Inhibition of binding of JAR 3 to factor H–binding protein (fHBP) by JAR 4 (50 μg/mL), as measured by ELISA. B, Inhibition of binding of JAR 4 by JAR 3 (50 μg/mL). JW-A2, negative control anti-meningococcal group A capsular monoclonal antibody; JW-C2, negative control anti-meningococcal group C capsular monoclonal antibody; Rab anti-fHBP and Rab pre-imm, 1:10 dilutions of serum from a rabbit after and before immunization with recombinant fHBP, respectively.