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fH-Dependent Complement Evasion by Disease-Causing Meningococcal Strains with Absent fHbp Genes or Frameshift Mutations*

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Abstract

Meningococci bind human fH to down-regulate complement, which enhances survival of the bacteria in serum. A major fH ligand is the vaccine candidate, factor H-binding protein (fHbp). Although fHbp has been considered an essential meningococcal virulence factor, rarely, invasive isolates with absent fHbp genes or frameshift mutations have been identified. In previous studies fH binding to these isolates was not detected. The aim of the present study was to investigate fH binding and complement evasion by invasive meningococcal serogroup B clinical isolates with absent fHbp genes or frameshift mutations. Four of the seven isolates tested bound human fH by flow cytometry and survived in IgG-depleted human serum. In all four, fH binding was decreased after inactivating the gene encoding NspA. Binding of fH to fHbp and NspA is specific for human fH. To investigate fH-dependent evasion of host defenses, human fH transgenic infant rats, or control littermates negative for human fH, were challenged IP with 10^3 to 10^4 CFU of two of the isolates with no detectable fH binding by flow cytometry. At six hours, bacteremia caused by both strains was higher in human fH transgenic rats than in control rats ($P < 0.002$). In conclusion, six of the seven isolates had evidence of fH binding and/or human fH-dependent complement evasion in transgenic rats. In four, NspA was as an alternative fH ligand. fHbp vaccination may select for mutants that do not require fHbp for complement evasion. Inclusion of additional target antigens in vaccines containing fHbp may delay emergence of these mutants.

Keywords

Neisseria meningitidis; vaccine; complement; factor H; fHbp; NspA; transgenic rat

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Conflict of interest. DMG and DMV are inventors on patents or patent applications in the area of meningococcal B vaccines. DMG also is principal investigator of laboratory research conducted on behalf of Children's Hospital Oakland Research Institute that is funded by grants from Novartis Vaccines and Diagnostics, and held a paid consultancy from Novartis. Serena Giuntini has no conflicts of interest.

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1. INTRODUCTION[†]

Serogroup B strains are responsible for ~30% of meningococcal disease in the United States [1], and 80 to 90% of disease in many European countries [2–4]. The serogroup B polysaccharide cross-reacts with sialylated proteins in some human tissues [5]. Vaccines that target this capsular polysaccharide risk eliciting auto-reactive antibodies. Alternative approaches for a vaccine against serogroup B strains target protein antigens. Among the most promising is factor H binding protein (fHbp), which is part of two vaccines in late stage clinical development [6–10]. One these vaccines (Bexero, Novartis Vaccines) recently was licensed in Europe [11].

fHbp binds the complement down-regulatory protein factor H (fH) [12]. Binding is specific for human fH [13], and is an important mechanism for survival of bacteria in serum [14, 15], and causing invasive disease [16]. Until recently, the gene for fHbp was found in all isolates tested from patients with invasive meningococcal disease [17–19]. Thus, fHbp was considered an important determinant of meningococcal virulence. During an extensive genotypic analysis of potential vaccine antigens among large collections of disease-causing meningococcal isolates, Lucidarme et al identified 18 serogroup B or C meningococcal strains in which fHbp genes were either absent, or contained frameshift mutations that resulted in non-functional fHbp molecules [20]. By far Western analysis, the investigators found no evidence of fH binding. Thus, the protein A mechanism by which these isolates evaded complement and caused disease in the absence of functional fHbp for recruiting fH was not identified, which is the subject of the present report.

2. METHODS

2.1. *Neisseria meningitidis* isolates

Lucidarme et al described 12 serogroup B and six serogroup C isolates with Δ T366 or Δ A650 frameshift mutations or absent fHbp genes [20]. Ten of the serogroup B isolates were from blood or CSF and two were nasopharyngeal isolates, which we excluded. For our studies, we selected seven of the invasive serogroup B isolates with representative multilocus sequence types (Table 1). Three had Δ T366 frameshift mutations, one had a Δ A650 frameshift mutation, and three had absent fHbp genes.

2.2. Sequences analysis of fHbp and porB genes

For each of the seven isolates, we confirmed the reported frameshift mutations or absent fHbp genes. The fHbp genes were amplified by PCR from heat-killed bacterial cells using the following oligonucleotides: 5'-CTGGCGGCTGCCGACTTCCTGATGGAA-3' and 5'-CCGTTATGCCAAGGGCGAATTGAACCAA-3', which amplified 500 bp upstream and downstream flanking regions of fHbp.

The PorB genes were amplified using PCR primers PBA1 and PBA2 previously described [21]. The identification of the porB alleles was established according to the *Neisseria* PubMLST sequence database site: <http://pubmlst.org/neisseria/porB/>. The correlation between allelic variants and serotypes was inferred using the scheme developed by Sacchi et al [22] and Abad et al [23] that defined the serotypes based on a combination of the sequences of the VRs.

[†]Abbreviations : fH, factor H; fHbp, factor H-binding protein; NspA, *Neisseria* surface

2.3. Creation of mutant strains with inactivated genes encoding *Neisseria* surface protein A (NspA)

NspA is a known second human fH ligand [24, 25]. To determine the effect of NspA on fH binding, we inactivated the genes encoding NspA by transforming the parent strain with a plasmid that contained a truncated *nspA* gene interrupted by a gene that conferred erythromycin resistance [26]. The resulting nonfunctional NspA genes were confirmed by sequencing of DNA obtained by PCR using the following primers: 5'-ACAGCAGGATCCTTTAACGGATTTC-3' and 5'-GTGGATGAAGCTTTGGACATTTC-3'.

2.4. Flow cytometric detection of binding of human fH or mouse antibodies to the surface of live *N. meningitidis* bacteria

The bacteria were grown in Mueller-Hinton broth (BD, Franklin Lakes, NJ) supplemented with 0.25% glucose to mid-log phase to an optical density at 620 nm (OD₆₂₀) of 0.6 [27]. Except where noted, the broth was supplemented with 0.02 mM cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-NANA; Sigma) to enhance sialylation of LOS [28]. For detection of serogroup B capsule, NspA or fHbp, we incubated ~10⁷ cells/ml for 1 h at room temperature with murine mAbs specific for the capsule (SEAM 12, 5 µg/ml [29]), NspA (14C7, 50 µg/ml [30]), or fHbp (JAR 41, 50 µg/ml, [31]). After washing the bacteria, the cells were incubated with Alexa Fluor 488 goat anti-mouse IgG (H+L) (Invitrogen), diluted 1:500, for 1 h at room temperature. The bacteria were washed twice with buffer as described [27], fixed with 0.5% (vol/vol) formaldehyde in PBS, and binding of the mAb was detected by flow cytometry.

For detection of human fH binding to the bacterial surface by flow cytometry, the bacterial cells were incubated for 1 hr at room temperature with ~90 µg/ml of fH as previously described [32].

2.5. Survival of bacteria in human serum

The bacterial cells were grown, harvested, and resuspended in buffer as described above for performing the flow cytometric assays. IgG was depleted from an adult human serum using protein G-sepharose as previously described [32]. Approximately 300 to 400 CFU of bacteria were added to wells of a microtiter plate (Nunclon Δ Surface; Thermo Fisher Scientific, Rochester, NY), which contained different dilutions of the serum (final concentrations of 15, 30 or 50%). The microtiter plates were incubated for 60 min at 37 °C in 5% CO₂ with agitation as previously described [27]. Colony counts were performed at time 0 and 60 mins. The percent survival was determined by comparing CFU of bacteria incubated with serum at T60 to that of bacteria incubated with serum that had been heated for 30 min at 56° C to inactivate complement.

2.6. Human fH-dependent survival of bacteria in infant rat serum

Binding of fH to fHbp is specific for human fH [13]. The ability of human fH to enhance survival of meningococci in infant rat serum can be used as a marker for the ability of the strain to utilize human fH to evade rat complement-mediated bacteriolysis [16]. In brief, pooled sera from 8- to 9-day-old wildtype Wistar rats (final concentrations of 20, 40 or 60%) together with 0, 10 or 100 µg/ml of purified human fH (Complement Technologies, Inc.) and ~400 CFU of bacteria were added to wells of microtiter plates (final volume, 40 µl), and the percentages of surviving bacteria after 60 mins incubation were determined as described above. In these experiments, there was no effect of the addition of human fH to the infant rat serum on hemolytic complement activity as measured with sensitized sheep red blood cells (EZ complement cells, Diamedix).

2.7. Rat C3b deposition on *N. meningitidis*

We used flow cytometry to measure the effect of human fH on deposition of rat C3b on the surface of live bacteria. The assay was performed as previously described [27] except that infant rat serum was used instead of human serum and we measured rat C3b with fluorescein isothiocyanate-conjugated goat anti-rat C3 antibody (MP Biomedical), which reacts with rat C3b and C3c.

2.8. Bacteremia in human fH transgenic infant rat

Details of the human fH transgenic infant rat model of meningococcal bacteremia have been previously described [16]. Rats aged 6 to 7 days were challenged i.p. with 100 μ l of a suspension containing $\sim 4 \times 10^2$ to 1×10^3 CFU of bacteria. Quantitative blood cultures were obtained at 6 h, which were performed as previously described [33].

2.9. Ethics statement

Coded isolates with absent fHbp genes or frameshift were provided by Dr. Ray Borrow, Manchester UK. Permission to use these isolates was obtained from the Children's Hospital Oakland Institutional Review Board (IRB). The human complement source was serum from an adult who participated in a protocol that was approved by the IRB. Written informed consent was obtained from the subject.

3. RESULTS

3.1. *Neisseria meningitidis* group B strains with absent fHbp genes or frameshift mutations can bind human fH

By flow cytometry none of the seven isolates bound with an anti-fHbp mAb JAR 41 (Figure 1, Panel B) that recognizes all fHbp sequence variants tested from sub-family A and B [31]. Although not shown, all seven isolates also were negative for binding to the anti-fHbp mAb by a more sensitive dot blot assay, which was performed as previously described [20]. Despite absent or non-functional fHbp molecules, four of the seven isolates showed fH binding above background by flow cytometry (strain 2 with a Δ T366 frameshift mutation, and strains 5, 6 and 7 with absent fHbp genes, Panel D). In one of the four (strain 5, which lacked a fHbp gene), fH binding was similar to that of the positive control serogroup B strain H44/76 (strain 8), which is a naturally high expresser of full length fHbp [14]. NspA, which is a second known fH ligand, was detected by flow cytometry in all four fH-binding isolates by an anti-NspA mAb (Panel C). One additional fH-negative isolate was positive for NspA binding (strain 3). Thus, while all fH-positive isolates were positive for surface-accessible NspA, binding of the anti-NspA mAb did not always correlate with fH binding. This disparity may relate to previous observations that other cell surface molecules such as longer LOS structures or decreased sialylation can interfere with binding of fH to NspA [24, 25]. Inactivating the NspA genes decreased binding of the anti-NspA mAb to background levels (Panel C), and, in the four strains positive for fH binding by flow cytometry, also decreased but did not completely eliminate fH binding (especially seen in strains 5 and 7, Panel D, black dashed lines).

3.2. *Neisseria meningitidis* group B strains with absent fHbp genes or frameshift mutations can survive in human serum

Despite lack of fHbp expression, six of the seven isolates survived in up to 50% IgG-depleted human serum (the highest concentration tested) when incubated at 37° for one hr (Figure 2). The exceptional isolate, strain 3, had a lower amount of capsular polysaccharide (Figure 1, Panel A), which likely accounted for poor survival in human serum. Among the six isolates with higher amounts of group B capsule, knocking out NspA expression did not

affect bacterial survival in IgG-depleted human serum when compared to survival of the respective WT isolate (Figure 2, dashed lines). Thus, while NspA contributed to binding of fH, expression of this molecule or fHbp was not required for survival in human serum.

3.3. Isolates with fHbp frameshift mutations, which were negative for human fH binding by flow cytometry, show evidence of human fH-dependent complement evasion in infant rat serum

Binding of fH to fHbp or NspA is specific for human fH [13, 25]. Thus the ability of human fH to regulate rat C3b deposition on the bacterial surface or increase survival of meningococci in rat serum can be used as markers of human-fH dependent down-regulation of complement activation [16]. We investigated rat C3b deposition and survival in infant rat serum of two of the strains with fHbp gene frameshift mutations, which were negative for human fH binding by flow cytometry (Strains 1 and 4). For C3b deposition we tested 40% infant rat serum: C3 deposited on both strains was down-regulated by the addition of human fH (Figure 3, Panel A). For bacterial survival we tested infant rat serum concentrations of 20, 40 and 60%. In the absence of added human fH, Strain 1 was more resistant to killing than Strain 4 (~50% survival in 60% infant rat serum, compared to ~5% survival for Strain 1, Figure 3, Panel B). The addition of 10 or 100 µg/ml of purified human fH increased survival of both strains.

In infant rat challenge experiments, human fH-positive rats or control littermates negative for human fH were challenged with ~1000 CFU of strain 1 or ~400 CFU of strain 4. At 6 hours, bacteremia for both strains was higher in the human fH-positive rats than in the respective control littermates negative for human fH ($P < 0.002$, Figure 3, Panel C). These data provided additional evidence of a human fH-dependent mechanism other than binding of fH to fHbp, which allowed these strains to recruit human fH, down-regulate complement, and cause bacteremia in the infant rats.

3.4. Effect of LOS sialylation on human fH-dependent complement evasion in infant rat serum

LOS sialylation recently was reported to down-regulate the alternative complement pathway in the absence of both fHbp and NspA [24], and some meningococcal strains require exogenous CMP-NANA for sialylation of LOS [28]. The experiments described above were performed with bacteria grown in broth supplemented with CMP-NANA to enhance LOS sialylation [34, 35], and to mimic conditions in vivo where CMP-NANA may be scavenged from physiological body fluids. We therefore compared the effect of human fH on survival of strains 1 and 4 in infant rat serum when the bacteria were grown in broth culture with or without CMP-NANA. Survival of Strain 1 in infant rat serum was not affected by CMP-NANA in the culture media (Figure 4). In contrast, without CMP-NANA, Strain 4 was more susceptible to killing by infant rat serum and required more human fH to increase survival than when grown with CMP-NANA.

4. DISCUSSION

Complement-dependent bactericidal activity is the main host defense against developing meningococcal disease (Reviewed in [36, 37]). *N. meningitidis* has evolved a number of mechanisms to evade complement-mediated bacteriolysis, including elaboration of polysaccharide capsules, lipooligosaccharide structure and sialylation [24, 37], and recruitment of the complement down-regulating molecule, factor H (fH) [12, 38]. Binding of fH to the bacterial surface leads to inactivation of C3b (to iC3b), and degradation of the C3bBb (the C3 convertase of the alternative pathway) through an interaction between fH

and factor I [39, 40]. Both mechanisms result in decreased formation of the membrane attack complex, and decreased bacteriolysis.

In 2009 Murphy et al reported a single serogroup B strain with a fHbp frameshift mutation in a surveys of disease-causing serogroup collections [19]. With that exception, until the report of Lucidarme et al in 2011 [20], full length genes encoding fHbp had been found in all invasive serogroup B strains tested [17, 19, 41]. Further, mutant strains in which the genes encoding fHbp had been inactivated were reported to be killed by normal human serum or blood [42]. Thus, fHbp had been considered an essential determinant of meningococcal virulence [42]. Early on, however, there were inconsistencies in the data: for example, some meningococcal fHbp knock-out mutants were reported to survive in human serum or blood [14] and, in 2010, Lewis et al reported that NspA in some meningococcal strains served as a human fH ligand [25].

We investigated fH binding and survival in human serum of seven serogroup B isolates from the Lucidarme study that lacked genes encoding a functional fHbp [20]. Three of the isolates were from the hypervirulent clonal complex ST 11, while the remaining four were from clonal complexes rarely associated with invasive meningococcal disease (Table 1). It is not known whether the patients infected with these isolates had underlying host abnormalities. In our studies, however, six of the seven isolates survived in IgG-depleted human serum, and the two strains used to challenge human fH transgenic rats caused bacteremia. Thus, with the exception of strain 3 with a $\Delta T366$ fHbp frameshift mutation, which was killed by human serum and had low amounts of capsule, the remaining isolates appeared to be fully pathogenic.

We detected fH binding by flow cytometry to all three of serogroup B isolates with absent fHbp genes, and in one of the four isolates with frameshift mutations (Figure 1). These strains had been reported by Lucidarme to be negative for fH binding by Far Western analyses. The Far Western analysis likely was insensitive for detection of fH binding because of disruption of fH complexes by the heat treatment and/or detergent solubilization steps required for the assay. Two of the isolates that were negative for fH binding by flow cytometry (strains 1 and 4) showed human fH-dependent down-regulation of rat complement-mediated C3b deposition and bacteriolysis, and enhanced bacteremia in human fH transgenic rats. The flow cytometric assay is not highly sensitive for detection of fH binding, particularly if the affinity of fH binding to the ligand is low. In humans, fH is present in serum at concentrations of 200–500 $\mu\text{g/ml}$ [32], and even low-affinity interactions between fH and bacterial ligands can result in complement inhibition [43, 44].

Inactivating the NspA gene in all four isolates that were positive for fH binding by flow cytometry decreased fH binding. These results are consistent with previous findings that NspA can serve as second meningococcal ligand for recruiting human fH [24, 25]. While the initial NspA data suggested that fH binding on intact organisms was best detected by flow cytometry assays in the absence of a capsule and/or presence of short chain (L8 immunotype) lipooligosaccharide (LOS) [25], more recent studies underscored the importance of NspA as a functional fH ligand on encapsulated strains with long (L3 immunotype) LOS when expression of fHbp was low [24], or antibodies to fHbp were present that inhibited binding of fH to fHbp [45]. Note that in our study all six isolates that survived in human serum also survived in human serum after inactivation of genes encoding NspA. These results are consistent with previous suggestions that there are additional mechanisms for meningococcal fH binding other than fHbp or NspA [16].

In the present study, we primarily investigated fH interactions with meningococci grown in broth supplemented with CMP-NANA, which is known to enhance LOS sialylation [24, 28].

When we compared survival in infant rat serum of bacteria grown with or without CMP-NANA, one of the two strains tested had increased susceptibility to killing by infant rat serum when grown without CMP-NANA and the addition of human fH had less effect on increasing serum resistance (Strain 4, Figure 4). Thus, this strain required exogenous CMP-NANA for resistance to killing by infant rat serum, which together with functional binding of fH to NspA and other putative fH ligands that remain undefined, contributed to down-regulation of bactericidal activity.

Collectively, our results indicate that fHbp is not essential for meningococcal pathogenesis. However, the 17 isolates with absent fHbp genes or frameshift mutations identified by the Lucidarme study represent only a tiny fraction of all disease-causing meningococcal isolates screened from the United Kingdom. Further, all of the mutant isolates were from one of three clonal complexes, cc11, cc286 or cc162, which infrequently are associated with serogroup B disease [20]. Thus, the rare occurrence of serogroup B strains with absent fHbp genes or frameshift mutations will have little overall effect on decreasing efficacy of fHbp vaccines. Immune pressure from widespread fHbp vaccination, however, could select for fHbp escape-mutants, which do not require this ligand for fH binding and evasion of host defenses. Use of vaccines that target multiple antigenic in addition to fHbp might delay emergence of these mutants.

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References

1. Cohn AC, MacNeil JR, Harrison LH, Hatcher C, Theodore J, Schmidt M, et al. Changes in *Neisseria meningitidis* disease epidemiology in the United States, 1998–2007: implications for prevention of meningococcal disease. *Clin Infect Dis*. 2010; 50:184–91. [PubMed: 20001736]
2. Campbell H, Andrews N, Borrow R, Trotter C, Miller E. Updated postlicensure surveillance of the meningococcal C conjugate vaccine in England and Wales: effectiveness, validation of serological correlates of protection, and modeling predictions of the duration of herd immunity. *Clin Vaccine Immunol*. 2010; 17:840–7. [PubMed: 20219881]
3. Trotter CL, Ramsay ME. Vaccination against meningococcal disease in Europe: review and recommendations for the use of conjugate vaccines. *FEMS Microbiol Rev*. 2007; 31:101–7. [PubMed: 17168998]
4. Trotter CL, Chandra M, Cano R, Larrauri A, Ramsay ME, Brehony C, et al. A surveillance network for meningococcal disease in Europe. *FEMS Microbiol Rev*. 2007; 31:27–36. [PubMed: 17168995]
5. Finne J, Leinonen M, Makela PH. Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet*. 1983; 2:355–7. [PubMed: 6135869]
6. Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, et al. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *JAMA*. 2012; 307:573–82. [PubMed: 22318278]
7. Snape MD, Dawson T, Oster P, Evans A, John TM, Ohene-Kena B, et al. Immunogenicity of two investigational serogroup B meningococcal vaccines in the first year of life: a randomized comparative trial. *Pediatr Infect Dis J*. 2010; 29:e71–9. [PubMed: 20844462]

8. Findlow J, Borrow R, Snape MD, Dawson T, Holland A, John TM, et al. Multicenter, open-label, randomized phase II controlled trial of an investigational recombinant meningococcal serogroup B vaccine with and without outer membrane vesicles, administered in infancy. *Clin Infect Dis*. 2010; 51:1127–37. [PubMed: 20954968]
9. Richmond PC, Marshall HS, Nissen MD, Jiang Q, Jansen KU, Garces-Sanchez M, et al. Safety, immunogenicity, and tolerability of meningococcal serogroup B bivalent recombinant lipoprotein 2086 vaccine in healthy adolescents: a randomised, single-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis*. 2012
10. Marshall HS, Richmond PC, Nissen MD, Jiang Q, Anderson AS, Jansen KU, et al. Safety and Immunogenicity of a Meningococcal B Bivalent rLP2086 Vaccine in Healthy Toddlers Aged 18 to 36 Months: A Phase 1 Randomized Controlled Clinical Trial. *Pediatr Infect Dis J*. 2012
11. Granoff DM. European Medicines Agency Recommends Approval of a Broadly Protective Vaccine against Serogroup B Meningococcal Disease. *Pediatr Infect Dis J*. 2012
12. Madico G, Welsch JA, Lewis LA, McNaughton A, Perlman DH, Costello CE, et al. The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance. *J Immunol*. 2006; 177:501–10. [PubMed: 16785547]
13. Granoff DM, Welsch JA, Ram S. Binding of complement factor H (fH) to *Neisseria meningitidis* is specific for human fH and inhibits complement activation by rat and rabbit sera. *Infection and immunity*. 2009; 77:764–9. [PubMed: 19047406]
14. Welsch JA, Ram S, Koeberling O, Granoff DM. Complement-dependent synergistic bactericidal activity of antibodies against factor H-binding protein, a sparsely distributed meningococcal vaccine antigen. *J Infect Dis*. 2008; 197:1053–61. [PubMed: 18419542]
15. Welsch JA, Ram S. Factor H and Neisserial pathogenesis. *Vaccine*. 2008; 26 (Suppl 8):I40–5. [PubMed: 19388163]
16. Vu DM, Shaughnessy J, Lewis LA, Ram S, Rice PA, Granoff DM. Enhanced bacteremia in human factor H transgenic rats infected by *Neisseria meningitidis*. *Infection and immunity*. 2012; 80:643–50. [PubMed: 22104107]
17. Masignani V, Comanducci M, Giuliani MM, Bambini S, Adu-Bobie J, Arico B, et al. Vaccination against *Neisseria meningitidis* using three variants of the lipoprotein GNA1870. *J Exp Med*. 2003; 197:789–99. [PubMed: 12642606]
18. Beernink PT, Leipus A, Granoff DM. Rapid genetic grouping of factor H-binding protein (genome-derived neisserial antigen 1870), a promising group B meningococcal vaccine candidate. *Clin Vaccine Immunol*. 2006; 13:758–63. [PubMed: 16829612]
19. Murphy E, Andrew L, Lee KL, Dilts DA, Nunez L, Fink PS, et al. Sequence diversity of the factor H binding protein vaccine candidate in epidemiologically relevant strains of serogroup B *Neisseria meningitidis*. *J Infect Dis*. 2009; 200:379–89. [PubMed: 19534597]
20. Lucidarme J, Tan L, Exley RM, Findlow J, Borrow R, Tang CM. Characterization of *Neisseria meningitidis* Isolates That Do Not Express the Virulence Factor and Vaccine Antigen Factor H Binding Protein. *Clin Vaccine Immunol*. 2011; 18:1002–14. [PubMed: 21508163]
21. Pajon R, Beernink PT, Granoff DM. Design of meningococcal factor h binding protein mutant vaccines that do not bind human complement factor h. *Infection and immunity*. 2012; 80:2667–77. [PubMed: 22615247]
22. Sacchi CT, Lemos AP, Whitney AM, Solari CA, Brandt ME, Melles CE, et al. Correlation between serological and sequencing analyses of the PorB outer membrane protein in the *Neisseria meningitidis* serotyping system. *Clin Diagn Lab Immunol*. 1998; 5:348–54. [PubMed: 9605990]
23. Abad R, Alcalá B, Salcedo C, Enriquez R, Uria MJ, Diez P, et al. Sequencing of the porB gene: a step toward a true characterization of *Neisseria meningitidis*. *Clin Vaccine Immunol*. 2006; 13:1087–91. [PubMed: 17028211]
24. Lewis LA, Carter M, Ram S. The relative roles of factor h binding protein, neisserial surface protein a, and lipooligosaccharide sialylation in regulation of the alternative pathway of complement on meningococci. *J Immunol*. 2012; 188:5063–72. [PubMed: 22504643]
25. Lewis LA, Ngampasutadol J, Wallace R, Reid JE, Vogel U, Ram S. The meningococcal vaccine candidate neisserial surface protein A (NspA) binds to factor H and enhances meningococcal resistance to complement. *PLoS Pathog*. 2010; 6:e1001027. [PubMed: 20686663]

26. Moe GR, Zuno-Mitchell P, Lee SS, Lucas AH, Granoff DM. Functional activity of anti-Neisserial surface protein A monoclonal antibodies against strains of *Neisseria meningitidis* serogroup B. *Infection and immunity*. 2001; 69:3762–71. [PubMed: 11349041]
27. Giuntini S, Reason DC, Granoff DM. Complement-mediated bactericidal activity of anti-factor H binding protein monoclonal antibodies against the meningococcus relies upon blocking factor H binding. *Infection and immunity*. 2011; 79:3751–9. [PubMed: 21708990]
28. Mandrell RE, Kim JJ, John CM, Gibson BW, Sugai JV, Apicella MA, et al. Endogenous sialylation of the lipooligosaccharides of *Neisseria meningitidis*. *Journal of bacteriology*. 1991; 173:2823–32. [PubMed: 1708379]
29. Granoff DM, Bartoloni A, Ricci S, Gallo E, Rosa D, Ravenscroft N, et al. Bactericidal monoclonal antibodies that define unique meningococcal B polysaccharide epitopes that do not cross-react with human polysialic acid. *J Immunol*. 1998; 160:5028–36. [PubMed: 9590252]
30. Moe GR, Zuno-Mitchell P, Hammond SN, Granoff DM. Sequential immunization with vesicles prepared from heterologous *Neisseria meningitidis* strains elicits broadly protective serum antibodies to group B strains. *Infection and immunity*. 2002; 70:6021–31. [PubMed: 12379678]
31. Vu DM, Pajon R, Reason DC, Granoff DM. A Broadly Cross-Reactive Monoclonal Antibody Against an Epitope on the N-terminus of Meningococcal fHbp. *Sci Rep*. 2012; 2:341. [PubMed: 22461972]
32. Beernink PT, Shaughnessy J, Braga EM, Liu Q, Rice PA, Ram S, et al. A meningococcal factor H binding protein mutant that eliminates factor H binding enhances protective antibody responses to vaccination. *J Immunol*. 2011; 186:3606–14. [PubMed: 21325619]
33. Welsch JA, Granoff D. Naturally acquired passive protective activity against *Neisseria meningitidis* Group C in the absence of serum bactericidal activity. *Infection and immunity*. 2004; 72:5903–9. [PubMed: 15385492]
34. Tsang RS, Law DK, Tsai C, Ng L. Detection of the *lst* gene in different serogroups and LOS immunotypes of *Neisseria meningitidis*. *FEMS microbiology letters*. 2001; 199:203–6. [PubMed: 11377868]
35. Smith H, Parsons NJ, Cole JA. Sialylation of neisserial lipopolysaccharide: a major influence on pathogenicity. *Microbial pathogenesis*. 1995; 19:365–77. [PubMed: 8852278]
36. Borrow R, Carlone GM, Rosenstein N, Blake M, Feavers I, Martin D, et al. *Neisseria meningitidis* group B correlates of protection and assay standardization--international meeting report Emory University, Atlanta, Georgia, United States, 16–17 March 2005. *Vaccine*. 2006; 24:5093–107. [PubMed: 16838413]
37. Schneider MC, Exley RM, Ram S, Sim RB, Tang CM. Interactions between *Neisseria meningitidis* and the complement system. *Trends Microbiol*. 2007; 15:233–40. [PubMed: 17398100]
38. Schneider MC, Exley RM, Chan H, Feavers I, Kang YH, Sim RB, et al. Functional significance of factor H binding to *Neisseria meningitidis*. *J Immunol*. 2006; 176:7566–75. [PubMed: 16751403]
39. Lu D, Silhan J, Macdonald JT, Carpenter EP, Jensen K, Tang CM, et al. Structural basis for the recognition and cleavage of abasic DNA in *Neisseria meningitidis*. *Proc Natl Acad Sci U S A*. 2012
40. Quattroni P, Li Y, Lucchesi D, Lucas S, Hood DW, Herrmann M, et al. Galectin-3 binds *Neisseria meningitidis* and increases interaction with phagocytic cells. *Cellular microbiology*. 2012
41. Fletcher LD, Bernfield L, Barniak V, Farley JE, Howell A, Knauf M, et al. Vaccine potential of the *Neisseria meningitidis* 2086 lipoprotein. *Infection and immunity*. 2004; 72:2088–100. [PubMed: 15039331]
42. Seib KL, Serruto D, Oriente F, Delany I, Adu-Bobie J, Veggi D, et al. Factor H-binding protein is important for meningococcal survival in human whole blood and serum and in the presence of the antimicrobial peptide LL-37. *Infection and immunity*. 2009; 77:292–9. [PubMed: 18852235]
43. Seib KL, Brunelli B, Brogioni B, Palumbo E, Bambini S, Muzzi A, et al. Characterization of diverse subvariants of the meningococcal factor H (fH) binding protein for their ability to bind fH, to mediate serum resistance, and to induce bactericidal antibodies. *Infection and immunity*. 2011; 79:970–81. [PubMed: 21149595]

44. Dunphy KY, Beernink PT, Brogioni B, Granoff DM. Effect of factor H-binding protein sequence variation on factor H binding and survival of *Neisseria meningitidis* in human blood. *Infection and immunity*. 2011; 79:353–9. [PubMed: 21041484]
45. Giuntini S, Reason DC, Granoff DM. Combined roles of human IgG subclass, alternative complement pathway activation, and epitope density in the bactericidal activity of antibodies to meningococcal factor h binding protein. *Infection and immunity*. 2012; 80:187–94. [PubMed: 22064712]
46. Gray SJ, Trotter CL, Ramsay ME, Guiver M, Fox AJ, Borrow R, et al. Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit. *J Med Microbiol*. 2006; 55:887–96. [PubMed: 16772416]

Research highlights

Meningococci with absent fHbp genes rarely can cause clinical disease

Naturally fHbp-deficient mutants can bind fH via alternative ligands such as NspA

Strains resist human complement-mediated bacteriolysis

Cause bacteremia in human fH transgenic infant rats

fHbp is not essential for meningococcal virulence

Figure 1. Flow cytometric studies

Panel A. Binding of group B anticapsular mAb (SEAM 12, 5 $\mu\text{g/ml}$). Solid black lines, WT isolates with anticapsular mAb; gray filled areas, WT bacteria without mAb. Numbers within panels are mean fluorescence intensities. **Panel B. Binding of anti-fHbp mAb (JAR 41, 50 $\mu\text{g/ml}$).** Solid black lines, WT isolates with anti-fHbp mAb; gray filled areas, WT bacteria without mAb. **Panel C. Binding of anti-NspA mAb (14C7, 50 $\mu\text{g/ml}$).** Solid black lines, WT isolates with anti-NspA mAb; dashed black lines, respective NspA KO mutants; gray filled areas, WT bacteria without mAb. **Panel D. Binding of fH to live bacterial cells.** Solid black line, human fH ($\sim 90 \mu\text{g/ml}$ in 20% IgG-depleted human serum) binding to wildtype (WT) strains; dashed black lines, human fH binding to NspA KO strains; gray filled areas, WT bacteria without human fH.

Figure 2. Survival of group B *Neisseria meningitidis* isolates in human serum

Bacterial cells were incubated with different percentages of IgG-depleted human serum for 60 min at 37 °C. Percent survival was compared to CFU/ml of bacteria incubated with serum that had been heated for 30 min at 56 °C to inactivate complement. Triangles with solid lines, WT isolates from patients. Circles with dashed lines, NspA KO mutants. Data were replicated in 2 or 3 independent experiments.

Figure 3. Effect of purified human fH on down-regulation of rat complement activation
Panel A. C3b deposition on *N. meningitidis* as measured by flow cytometry. Bacteria were incubated at RT for 15 min in 40% pooled sera from 8 to 9 days old rats in the presence of 0 (gray line) or 100 $\mu\text{g/ml}$ (black line) of human fH. Data were replicated in 2 independent experiments. **Panel B. Survival of *N. meningitidis* incubated for 60 mins at 37 C in infant rat serum.** Gray filled boxes with solid line, 0 $\mu\text{g/ml}$ of human fH; open circles with dotted line, 10 $\mu\text{g/ml}$; open triangles with solid line, 100 $\mu\text{g/ml}$. The data points represent median values from triplicate measurements. Error bars represent ranges. The results were replicated in a separate independent experiment (data not shown). **Panel C. Meningococcal bacteremia in human fH transgenic rats.** Six- to seven-day-old animals were challenged i.p. with ~ 1000 CFU of strain 1 or ~ 200 CFU of strain 4. Blood cultures were obtained at 6 h after the challenge. Open circles, CFU/ml of individual human fH-positive animals; gray filled boxes, CFU/ml of individual human fH-negative animals. The geometric means of the respective CFU/ml were higher in human fH-positive than in human fH-negative animals ($p=0.0006$ for strain 1; $p=0.002$ for strain 2).

Figure 4. Effect of CMP-NANA on human fH-dependent survival in infant rat serum

Bacteria grown in broth culture with or without supplemental CMP-NANA were washed and incubated at 37 C for 60 mins in pooled infant rat serum in the presence of 0, 10 or 100 $\mu\text{g/ml}$ of purified human fH. The respective survival at different concentrations of human fH was similar for Strain 1 when grown with or without CMP-NANA. Strain 4 grown without CMP-NANA was more susceptible to killing by infant rat serum, and showed less effect of human fH on enhancing survival than when grown with CMP-NANA. Data for each strain were replicated in 2 or 3 independent experiments

Neisseria meningitidis
serogroup B strains.

Table 1

Strain No	Original Strain designation [¶]	Site	Multilocus Sequence Type [§]	fHbp Gene	fHbp Variant (v.) group	PorA Sero-subtype ^{**}	Closest PorB protein [†]	fH Binding by Flow Cytometry ^{††}
1	M08-240254	Blood	11	ΔT366	v.1 (78)	1.5	1 (Class 2)	–
2	M05-240072	Blood	11	ΔT366	v.1 (Not designated [*])	1.5	1 (Class 2)	+
3	M06-241270	Blood	11	ΔT366	v.1 (78)	1.5	1 (Class 2)	–
4	M08-240219	CSF	162	ΔA650	v.2 (21)	1.14	149 (Class 3)	–
5	M01-241604	Blood	3009	Not Detected	–	NT ^{**}	230 (Class 2)	+++
6	M01-242298	Blood	286	Not Detected	–	1.16	192 (Class 2)	+
7	M01-242525	Blood	1867	Not Detected	–	1.16	192 (Class 2)	++
8	H44/76 (positive control)	Blood	32	Full length	v.1 (1)	1.7	101 (Class 3)	+++

[¶]As described by Lucidarme et al [20]. The positive control H44/76, was from an epidemic strain from Norway and known to be a high expresser of fHbp [14].

[§]Inferred from the DNA sequence after correction of the frameshift mutation. Variant group and fHbp ID assignments are described on the public website, <http://pubmlst.org/neisseria/>

^{*} 99% identical to fHbp ID 2

^{**} Based on reactivity with specific anti-PorA mAbs [46]; NT, not reactive

[†] Based on inferred amino acids from gene sequencing and comparison with PorB public database, <http://pubmlst.org/neisseria>

^{††} Live bacteria: +, 4x-fold above negative control cells without added fH; ++, 75 to 100% binding of fH compared to that of the positive control strain H44/76 +++>125% binding of fH compared to that of positive control