Improving Safety and Efficacy of Meningococcal Vaccines

Vaccines based on a relatively sparse surface lipoprotein extend coverage to serogroup B; amino acid changes could lead to further enhancements

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The gram-negative bacterium Neisseria meningitidis, which causes sepsis and meningitis, erupts periodically in Sub-Saharan Africa, where 100,000 cases can arise within a few months. While meningococcal epidemics in the United States and Europe last arose in the 1940s, endemic disease in these regions currently leads to about 10,000 cases per year. Despite effective antibiotics, case fatality rates range from about 10 to 15%, and serious infections can lead to neurological damage, deafness, or limb amputations. Control of meningococcal disease, therefore, is a public health imperative, which is best achieved by vaccination.

Capsular Polysaccharide Is the Basis for All but Serogroup B Vaccines

N. meningitidis, which is limited to humans, is found with 12 different capsular polysaccharide structures, five of which—serogroups A, B, C, W, and Y—are responsible for causing nearly all disease in immunologically normal hosts living in industrialized countries. Safe and effective polysaccharide-protein conjugate vaccines are available against four of these five capsular serogroups. In the United States, for example, quadrivalent A, C, Y, and W conjugate vaccines are recommended for all teenagers.

However, the conjugate vaccine approach has not worked against serogroup B N. meningitidis because its capsular polysaccharide is an autoantigen. Thus, the challenge for developing a vaccine to protect against serogroup B strains is to identify conserved noncapsular antigens expressed by most strains and capable of eliciting serum complement-mediated bactericidal antibodies, which are the hallmark of protection against this disease.

By the year 2000 and despite 25 years of efforts to develop such a vaccine, suitable candidates were not available. Since then, however, new approaches to antigen discovery, including genome mining and proteomics, led to considerable progress.

Antigen Discovery Leads to Development of Serogroup B Vaccine Candidates

Through genome mining, Vega Masignani and colleagues at Novartis Vaccines in Siena, Italy, in 2003 identified from N. meningitidis a 28-kDa lipoprotein, which they called genome-derived neisserial antigen (GNA) 1870; this designation was based on the gene number because the function of the protein was not known. This protein, which is found in nearly all meningococcal strains, induces cross-reactive serum bactericidal antibodies in mice. A year later, L. D. Fletcher and colleagues at Wyeth Vaccines, now part of Pfizer, which is headquartered in New York, N.Y., reported finding the same antigen, which they identified using membrane fractionation and called lipoprotein (LP) 2086.

SUMMARY

➤ Until recently, candidate vaccines against Neisseria meningitidis failed to protect against serogroup B strains.
➤ When used as an antigen, the relatively scarce surface lipoprotein, identified as being Factor H binding protein (FHbp), provides broad protection against serogroup B meningococcal disease.
➤ Although the scarcity of FHbp on the bacterial surface presents challenges when it comes to developing vaccines, many such challenges are being met.
➤ Immunizing with FHbp, which forms a complex with human complement Factor H (FH), can mask vaccine epitopes that are important for eliciting serum bactericidal antibody responses.
➤ Modifying specific amino acids in FHbp antigens, which decrease FH binding, might enhance vaccine efficacy and safety.
Based on amino acid sequence relatedness, the scientists at Wyeth described two subfamilies (A and B) for this antigen, while scientists at Novartis subdivided the antigen into three variant groups (Fig. 1A). There was general agreement that antibodies to this antigen from variant group
1 (sub-family B) were bactericidal only against strains from variant group 1 but not against strains with variant groups 2 or 3 (sub-family A), and vice versa.

Subsequently, my colleague Sanjay Ram, then at Boston University and now at the University of Massachusetts, Worcester, and I renamed this antigen Factor H binding protein (FHbp) after discovering that the antigen bound complement Factor H. Based on a phylogenetic analysis of 70 unique FHbp amino acid sequences, my colleague Peter Beernink at Children’s Hospital Oakland Research Institute (CHORI) in California and I proposed a modular system for classifying this antigen. We found that FHbp contains five variable segments, designated A, B, C, D, and E, each of which is flanked by short sequences of invariant residues. Each variable segment derives from one of two genetic lineages (Fig. 1B: lineage 1, red symbols, and lineage 2, white symbols).

Overall, approximately 57% of the FHbp amino acid sequence variants contain all five variable segments from one or the other lineage (for example, amino acid sequence variants ID 1 and 28 from variant groups 1 and 3, respectively [Fig. 1B]). The remaining 43% of the sequence variants are natural chimeras containing some segments derived from one lineage and other segments from the second (for example, ID 77 from variant group 2 [Fig. 1B]). One such chimeric protein has 96% amino acid identity with a FHbp ortholog in N. gonorrhoeae. These data suggest that recombination between N. meningitidis and N. gonorrhoeae FHbp progenitors generated a family of modular, antigenically diverse meningococcal FHbp proteins.

In general, the reactivity of anti-FHbp monoclonal antibodies (mAbs) is specific for segments derived from one or the other of those lineages, but not both. For example, mAbs JAR 1, 3, 4, and 5 and mAb502 react with epitopes present only on segments derived from lineage 1, while mAbs JAR 10, 11, 13, 31, 32, and 34 react only with epitopes on segments derived from lineage 2 (Fig. 1B). In the group shown, the only exception is JAR 41, which recognizes a conserved epitope located on the A segment at the N-terminal portion of the molecule that is present on all FHbp sequence variants tested to date.

**Scarcity of FHbp on the Bacteria Poses Vaccine Efficacy Challenges**

Bacterial vaccines usually target antigens that are relatively abundant on the surface of the pathogen. However, FHbp appears to be sparsely arrayed along the surface of meningococcal strains—explaining, in part, why the antigen remained unidentified for many years.

This relative scarcity of FHbp presents challenges when it comes to vaccine development.

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**FIGURE 2**

**Interaction between human FH and N. meningitidis.**

(A) Effect of human FH on survival of serogroup B strain H44/76 in infant rat serum (from Vu, D. M., J. Shaughnessy, L. A. Lewis, S. Ram, P. A. Rice, and D. M. Granoff, Infect. Immun. 80:643–650, 2012; reprinted with permission from the publisher). (B) Inhibition of binding of FH to N. meningitidis group B strain H44/76 by anti-FHbp mAbs as measured by flow cytometry. Dark gray filled area, bacteria without added FH or mAb as a negative control. Light orange-filled area, FH and bacteria without added anti-FHbp mAbs. Purple dashed lines, anti-FHbp mAb JAR 5, which inhibited FH binding; orange solid line, anti-FHbp mAb502, which did not inhibit FH binding (Adapted from Giuntini, S., D. C. Reason, and D. M. Granoff, Infect. Immun. 79:3751–759, 2011; reprinted with permission of publisher).
For example, with only rare exceptions, individual anti-FHbp mAbs are not bactericidal with human complement. In contrast, mixtures of anti-FHbp mAbs, which individually lack bactericidal activity, prove bactericidal when their respective epitopes are not overlapping.

For instance, anti-FHbp mAbs JAR 5 and JAR 4 recognize nonoverlapping epitopes (Fig. 1B). With human complement, neither mAb has bactericidal activity when tested individually. In contrast, combinations of the two mAbs are bactericidal when administered at concentrations less than 1 µg/ml.

The classical complement pathway requires that two appropriately spaced IgG molecules be bound to engage C1q. In other words, individual IgG mAbs binding to sparsely situated surface antigens do not efficiently engage C1q to activate the complement pathway, meaning there is little or no bacteriolysis. This shortcoming is particularly apparent if complement activation is down-regulated—for example, when a complement down-regulating molecule such as Factor H binds to the bacterial surface. In contrast, two or more mAbs binding to nonoverlapping epitopes can provide sufficient antibody density to engage C1q and activate complement-mediated bacteriolysis, particularly if at least one of the mAbs also inhibits binding of FH to FHbp (Fig 2B).

While those mAb epitope mapping and functional studies were under way, both Novartis and Pfizer began phase 1 vaccine safety and immunogenicity clinical trials in humans. The Pfizer vaccine includes two recombinant FHbp antigens, one from variant group 1 and the other from variant group 3 (Fig. 1A). Coverage against strains with variant group 2 depends on cross-reactivity with variant group 3.

The Novartis vaccine contains a recombinant FHbp from variant group 1. Its coverage of meningococcal strains with FHbp from variant groups 2 and 3 depends on two other recombinant proteins, called neisserial heparin binding antigen and NadA, as well as outer membrane vesicles. Both the Pfizer and Novartis candidate vaccines are well-tolerated and elicit complement-mediated serum bactericidal antibody responses. In December 2012, the Novartis vaccine, called Bexsero®, was approved by regulatory agencies in the European Union and, subsequently, in Australia and Canada, for use at 2 months of age or older. In 2014, the Pfizer vaccine, which targets adolescents, was in the late stages of clinical development in the United States and other countries.

**Delving More Deeply into FHbp Antigen Function**

One disadvantage of using genome mining or proteomics to identify vaccine antigens is that their functions may not be determined by the time clinical trials begin, which was the case for the Pfizer and Novartis candidate vaccines containing FHbp. In 2006, Sanjay Ram, his collaborators at Boston University, and my group reported that the FHbp antigen recruits a host complement down-regulatory protein called Factor H to the bacterial surface.

When meningococcal cells bind FH, they can evade complement. However, the binding of FH

![Figure 3](image-url)

**Figure 3**

Relationship between serum concentrations of human FH in human FH transgenic mice and serum bactericidal antibody titers to a recombinant FHbp vaccine that bound human FH. The left side of the figure shows the titers of individual wild-type (WT) mice (human FH <12.5 µg/ml); the right side of the figures shows the titers of individual transgenic mice in relation to serum human FH concentrations. In WT mice whose mouse FH did not bind to the FHbp vaccine, the median bactericidal titer after three doses of vaccine was 1:220. In the human FH transgenic mice, the higher the serum concentration of human FH, the lower the serum bactericidal titer (r = −0.65, P = 0.02). (Adapted from Beernink, P. T., J. Shaughnessy, E. M. Braga, Q. Liu, P. A. Rice, S. Ram, and D. M. Granoff, J. Immunol. 186:3606–3614, 2011; reprinted with permission.)
to FHbp is specific for human FH. Thus, rat, mouse, rabbit, and even some nonhuman primate versions of FH do not bind to the meningococcal FH binding protein, nor to a second subsequently discovered meningococcal FH ligand, neisserial surface protein A.

Because the binding protein specificity is for the human version of FH, many meningococcal strains that are highly pathogenic in humans and that resist killing by nonimmune human serum are rapidly killed when incubated in nonhuman serum (Fig. 2A). The addition of as little as 3 μg/ml of purified human FH to the infant rat serum rescues the organism (Fig. 2A). Moreover, some anti-FHbp mAbs can block binding of FH to FHbp if the antibodies are directed at epitopes in or near the FH combining site of FHbp. For example, anti-FHbp mAb JAR 5 blocks FH binding, but not anti-FHbp mAb 502 (Fig. 2B). For many strains, the ability of anti-FHbp antibodies to inhibit binding of FH to FHbp is important for enhancing anti-FHbp bactericidal antibody activity because, with less bound FH, the organism becomes more susceptible to complement-mediated bactericidal activity.

From the point of view of FHbp vaccine development, we suspected that, when humans are immunized with FHbp, the antigen would form a complex with human FH. Moreover, this binding might mask FHbp epitopes in the FH binding site that are important for eliciting serum bactericidal antibody responses.

With Peter Rice and Sanjay Ram at the University of Massachusetts and Peter Beernink at CHORI, we investigated this hypothesis by comparing serum antibody responses of wild-type and human FH transgenic mice immunized with a recombinant FHbp vaccine that binds human FH but not mouse FH. Compared to wild-type mice, the transgenic mice had normal serum IgG and bactericidal antibody responses to a control meningococcal serogroup C conjugate vaccine that did not bind FH.

In contrast, the transgenic mice had lower serum bactericidal antibody responses than did wild-type mice when immunized with a recombinant FHbp vaccine that binds human FH. Further, there was an inverse correlation between the concentrations of human FH in the sera of the transgenic mice and the serum bactericidal antibody titers elicited by the FHbp vaccine that binds human FH (Fig. 3). The effect of human FH on decreasing bactericidal antibody responses is significant in mice with serum human FH concentrations more than 250 μg/ml, which are present in normal human sera.

Modifying FHbp Antigens Might Enhance Vaccine Efficacy and Safety

Christoph Tang and colleagues then at the Imperial College in London, England, and now at Oxford University determined a crystal structure of FHbp in a complex with a fragment of human FH. They also engineered a mutant FHbp vaccine with decreased FH binding by replacing two FHbp glutamate residues, which mediated ion-pair interactions with FH, with alanine residues.

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**FIGURE 4**

Immunogenicity of native outer membrane vesicle vaccines with attenuated endotoxin and overexpressed wild-type (WT) or mutant FHbp. BALB/c human FH transgenic mice were immunized with NOMV vaccines prepared from mutants of serogroup B strains with attenuated endotoxin (LpxL1 knockout) and overexpressed (OE) wild-type FHbp that bound human FH, or a R41S mutant FHbp with 100-fold lower affinity for human FH, or a FHbp knockout (KO) mutant (negative control). For the OE WT and R41S mutants, each symbol represents the serum bactericidal titers of an individual mouse measured against serogroup B strain CU385 with a heterologous PorA to that of the vaccine and a FHbp sequence that matched that of FHbp in the wild-type FHbp vaccine. The titers in the FHbp KO and alum groups were each measured in two serum pools. The NOMV vaccine containing the mutant R41S FHbp elicited higher titers than the NOMV vaccine containing FHbp that bound human FH (P = 0.001) (from Beernink, P. T., J. Shaughnessy, R. Panama, E. M. Braga, S. Ram, and D. M. Granoff, PLoS Pathog. 8:e1002688, 2012; reprinted with permission.)
Although substituting alanine for glutamate does not appear to destabilize the molecule globally, we found that the mutations greatly decrease the immunogenicity of this altered protein in mice, in which neither the mutant nor the wild-type control FHbp vaccine binds FH.

With Peter Beernink and Rolando Pajon at CHORI, we engineered alternative mutant FHbp vaccines containing other single amino acid substitutions, and they decrease the binding of human FH by 30- to 100-fold while retaining vaccine immunogenicity. In experiments with human FH transgenic mice in which serum human FH concentrations were 250 μg/ml or greater, these FH “low-binding” mutant FHbp vaccines elicit significantly higher serum bactericidal antibody responses than do the control FHbp vaccines that bind human FH.

In one such experiment (Fig. 4), we compared the immunogenicity of native outer membrane vesicle (NOMV) vaccines prepared from meningococcal strains with genetically attenuated endotoxin (LpxL1 knockout) and overexpressed wild-type or mutant FHbp. In the mutant FHbp, an Arg at residue 41 was replaced with Ser (R41S), and this change decreased binding to human FH by 100-fold. In human FH transgenic mice, the geometric mean serum bactericidal titer to the mutant NOMV vaccine was 19-fold higher than to the NOMV vaccine containing FHbp that binds human fH (P = 0.001, Fig. 4). The serum bactericidal antibody responses measured against the meningococcal test strain were directed at FHbp based on serum antibody depletion experiments, and lack of serum bactericidal responses of control mice immunized with a NOMV FHbp knockout vaccine.

Vaccines containing FHbp are being introduced to large populations in Europe and other countries. Thus, the prospects of protecting against all meningococcal pathogens, including serogroup B strains, have never been better. Based on studies in human FH transgenic mice, the ability of FHbp vaccines to elicit protective antibodies in humans likely can be enhanced by substituting specific amino acids in FHbp to decrease human FH binding.

The mutant low-FH-binding FHbp vaccines also may be safer. Several human diseases, such as rheumatoid diseases and atypical hemolytic uremic syndrome, are associated with autoantibodies to FH. While autoantibodies to FH have not been reported in individuals immunized with FHbp vaccines, in theory, immunizing humans with a foreign bacterial antigen (FHbp) that forms a complex with a host protein (FH) poses a higher risk for eliciting anti-FH autoantibodies than would immunizing instead with a FH low- or non-binding mutant antigen. Determining the effects of binding of FH to FHbp vaccines on protective antibody responses and on vaccine safety in humans also will be important for investigators developing vaccines that target other virulence factors that bind host proteins in other microbial pathogens.

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Author’s Note

Dan Granoff is an inventor on patents and patent applications involving meningococcal B vaccines.

Suggested Reading


Granoff, D. M., S. Ram, and P. T. Beernink. 2013. Does binding of complement factor H to the meningococcal


