

**NON-NATURALLY OCCURRING FACTOR H BINDING PROTEINS
(FHBP) AND METHODS OF USE THEREOF**

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit of U.S. Provisional Patent Application Serial No. 61/861,662, Filed on August 2, 2013, the disclosure of which application is herein incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant no. R01 AI 099125-01 awarded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health. The government has certain rights in this invention.

INTRODUCTION

[0003] *Neisseria meningitidis* (*N. meningitidis*) is a Gram-negative bacterium which colonizes the human upper respiratory tract and is responsible for worldwide sporadic and cyclical epidemic outbreaks of, most notably, meningitis and sepsis. Infection and morbidity rates are highest in children under 2 years of age. Like other Gram-negative bacteria, *N. meningitidis* typically possess a cytoplasmic membrane, a peptidoglycan layer, an outer membrane which together with the capsular polysaccharide constitute the bacterial wall, and pili, which project into the outside environment. Encapsulated strains of *N. meningitidis* are a major cause of bacterial meningitis and septicemia in children and young adults. The prevalence of invasive *N. meningitidis* infections have driven the search for effective vaccines that can confer immunity across different strains, and particularly across genetically diverse group B strains with different serotypes or serosubtypes.

[0004] Factor H Binding Protein (fHbp, also referred to in the art as lipoprotein 2086 (Fletcher et al (2004) *Infect Immun* 72:2088-2100), Genome-derived Neisserial antigen (GNA) 1870 (Masignani et al. (2003) *J Exp Med* 197:789-99) or “741”) is a surface-exposed lipoprotein expressed in the *N. meningitidis* bacterium. fHbp binds to human complement factor H (fH), which down-regulates complement activation. Binding of fH to the bacterial surface is an important mechanism by which the pathogen survives in non-immune human

serum or blood and evades innate host defenses. Recently, genetic variation in the human factor H gene cluster was found to affect susceptibility to developing meningococcal disease (Davila S et al. (2010) Nat Genetics doi:10.1038/ng.640). Binding of fH to fHbp is specific for human fH and could account for why *Neisseria meningitidis* is strictly a human pathogen.

[0005] There remains a need for a fHbp polypeptide that can elicit effective bactericidal antibody responses.

SUMMARY

[0006] Non-naturally occurring factor H binding proteins derived from variant 3 fHbp that can elicit antibodies that are bactericidal for at least one strain of *N. meningitidis*, and methods of use of such proteins, are provided.

[0007] In certain embodiments, a non-naturally occurring factor H binding protein (fHbp) derived from a naturally occurring variant 3 fHbp is disclosed. The non-naturally fHbp may include a substitution of the histidine at position 223 of the naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan, wherein the numbering of position 223 is based on the numbering of the mature fHbp ID 1. The non-naturally occurring fHbp may have a lower affinity for human factor H (fH) than fHbp ID 79.

[0008] In certain embodiments, the histidine may be substituted with arginine.

[0009] In certain cases, the variant 3 fHbp may be a modular group V fHbp. In other cases, the variant 3 fHbp may be a modular group II fHbp.

[0010] Exemplary non-naturally occurring fHbp may include an amino acid sequence having at least 90% sequence identity to the amino acid sequence of the fHbp ID 79.

[0011] In certain cases, the non-naturally occurring fHbp may include the amino acid sequence of fHbp ID 28 with the amino acid substitution H223R.

[0012] In certain cases, the non-naturally occurring fHbp may include the amino acid sequence of fHbp ID 67 with the amino acid substitution H223R.

[0013] In certain cases, the non-naturally occurring fHbp may include the amino acid sequence of fHbp ID 175 with the amino acid substitution H223R.

[0014] In certain cases, the non-naturally occurring fHbp may include the amino acid sequence of fHbp ID 79 with the amino acid substitution H223R.

[0015] In certain cases, the non-naturally occurring fHbp may include the amino acid sequence of fHbp ID 45 with the amino acid substitution H223R.

[0016] In another embodiment, an immunogenic composition is provided. The immunogenic composition may include: a) the non-naturally occurring fHbp as disclosed herein; and b) a pharmaceutically acceptable excipient.

[0017] In certain cases, the non-naturally occurring fHbp may be expressed on surface of a vesicle preparation prepared from a *N. meningitidis* strain expressing the non-naturally occurring fHbp.

[0018] In certain cases, the non-naturally occurring fHbp may be present as an isolated polypeptide in the immunogenic composition.

[0019] In exemplary cases, pharmaceutically acceptable excipient may include an adjuvant.

[0020] In certain cases, the immunogenic composition may further include an additional *N. meningitidis* antigen.

[0021] A method of eliciting an antibody response in a mammal against *N. meningitidis* is provided. The method may involve administering to a mammal the non-naturally occurring fHbp disclosed herein, or the immunogenic composition as provided herein.

[0022] In certain cases, the administering provides for production of antibodies that are bactericidal against *N. meningitidis*.

[0023] A nucleic acid encoding the non-naturally occurring fHbp provided herein is also disclosed. Also provided herein is a recombinant expression vector that includes the nucleic acid encoding the non-naturally occurring fHbp. Exemplary embodiments include a genetically modified host cell that includes the nucleic acid encoding the non-naturally occurring fHbp or the recombinant expression vector that includes the nucleic acid encoding the non-naturally occurring fHbp.

[0024] Another immunogenic composition is disclosed herein. This immunogenic composition may include: a) a vesicle obtained from a genetically modified *Neisseria* host cell that is genetically modified with a nucleic acid encoding the non-naturally occurring fHbp according to the present disclosure, such that the encoded non-naturally occurring fHbp is produced by the genetically modified host cell, wherein the vesicle includes the encoded non-naturally occurring fHbp; and b) a pharmaceutically acceptable excipient.

[0025] In certain cases, the vesicle may be a native outer membrane vesicle.

[0026] In certain cases, the host cell may be genetically modified to provide for decreased or no activity of a polypeptide product of the *lpxL1* gene and/or the *lpxL2* gene.

[0027] In exemplary embodiments, the host cell is genetically modified to provide for increased expression of a Neisserial antigen.

[0028] A method of eliciting an antibody response against Neisseria in a mammal is provided. The method may include administering to a mammal the immunogenic composition that includes: a) a vesicle obtained from a genetically modified Neisseria host cell that is genetically modified with a nucleic acid encoding the non-naturally occurring fHbp according to the present disclosure, such that the encoded non-naturally occurring fHbp is produced by the genetically modified host cell, wherein the vesicle includes the encoded non-naturally occurring fHbp; and b) a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Figure 1. SDS-polyacrylamide gel indicating size and purity of recombinant fHbp ID 79 wild-type and mutants. Lane 1, Benchmark Ladder (Invitrogen); lane 2 fHbp ID 79 wild-type; lane 3 fHbp ID 79 H223R mutant; lane 4, fHbp ID 79 H223A mutant. 2 µg of each of the recombinant fHbps was loaded on the gel. Note that the amino acid numbering is based on the numbering of the mature fHbp ID 1. See Example 1 for details.

[0030] Figure 2. Binding of human factor H to fHbp ID 79 mutants H223A and H223R by ELISA. Panel A shows binding of human factor H. fHbp ID 79 wild-type (WT), circular symbols; fHbp ID 79 H223A mutant, triangular symbols; fHbp ID 79 H223R mutant, asterisk symbols. Panel B shows binding of a control mAb, JAR 11. Note that the amino acid numbering is based on the numbering of the mature fHbp ID 1. See Example 2 for details.

[0031] Figure 3. Binding of human factor H to fHbp ID 22 mutants T221A and H223A by ELISA. Panel A shows binding of human factor H. fHbp ID 22 wild-type (WT), circular symbols; fHbp ID 22 T221A mutant, asterisk symbols; fHbp ID 22 H223A mutant, triangular symbols. Panel B shows binding of a control mAb, JAR 13. Note that the numbering of T221 and H223 is based on the numbering of the mature fHbp ID 1. See Example 3 for details.

[0032] Figure 4. An alignment of amino acid sequence of mature fHbp ID1, fHbp ID 22, fHbp ID 79, and fHbp ID 28. H223 is indicated with an arrow.

[0033] Figure 5. An alignment of amino acid sequence of mature v. 3 fHbps. H223 is indicated with an arrow.

[0034] Figure 6. A schematic representation of fHbp modular groups I-IX.

[0035] Figure 7. A graph showing bactericidal responses of mice to fHbp ID 79 vaccines. fHbp ID 79 wild-type (WT) and fHbp ID 79 H223R mutant were tested. For the fHbp vaccines, each symbol represents the titer of an individual serum sample; for the aluminum (Alum) control group, each symbol represents the titer of a pool of three serum samples. The horizontal lines represent the geometric mean titer for each group.

[0036] Before the present invention and specific exemplary embodiments of the invention are described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0037] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

[0038] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to amino acid modifications, including amino acid substitutions, relative to a reference amino acid sequence are specifically embraced by the present invention and are disclosed herein just as if each and every combination were individually and explicitly disclosed, to the extent that such combinations embrace polypeptides having desired features, e.g., non-naturally occurring fHbp polypeptides having a lower affinity for a human fH than that of a naturally occurring fHbp. In addition, all sub-combinations of such amino acid modifications (including amino acid substitutions) listed in the embodiments describing such amino acid modifications are also specifically embraced by the present invention and are disclosed herein just as if each

and every such sub-combination of such amino acid modifications was individually and explicitly disclosed herein.

[0039] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0040] It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an antigen” includes a plurality of such antigens and reference to “the protein” includes reference to one or more proteins, and so forth.

[0041] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0042] As noted above, non-naturally occurring factor H binding proteins derived from variant 3 fHbp that can elicit antibodies that are bactericidal for at least one strain of *N. meningitidis*, and methods of use such proteins, are provided.

DEFINITIONS

[0043] “Factor H Binding Protein” (fHbp), which is also known in the literature as GNA1870, GNA 1870, ORF2086, LP2086 (lipoprotein 2086), and “741” refers to a class of *N. meningitidis* polypeptides. fHbp is found in nature as a lipoprotein expressed on the surface of *N. meningitidis* strains. fHbps have been sub-divided into three fHbp variant groups (referred to as variant 1 (v.1), variant 2 (v.2), and variant 3 (v.3) in some reports (Masignani et al. (2003) *J Exp Med* 197:789-99) and Family A and B in other reports (see, e.g., Fletcher et al. (2004) *Infect Immun* 72:2088-2100)) based on amino acid sequence variability and immunologic cross-reactivity (Masignani et al. (2003) *J Exp Med* 197:789-99). Each unique fHbp found in *N. meningitidis* is also assigned a fHbp peptide ID according

to neisseria.org or pubmlst.org/neisseria/fHbp/ website. Because the length of variant 2 (v.2) fHbp protein (from strain 8047, fHbp ID 77) and variant 3 (v.3) fHBP (from strain M1239, fHbp ID 28) differ by -1 and +7 amino acid residues, respectively, from that of MC58 (fHbp ID 1), the numbering used to refer to residues for v.2 and v.3 fHbp proteins differs from numbering based on the actual amino acid sequences of these proteins.

[0044] The term “heterologous” or “chimeric” refers to two components that are defined by structures derived from different sources or progenitor sequences. For example, where “heterologous” is used in the context of a chimeric polypeptide, the chimeric polypeptide can include operably linked amino acid sequences that can be derived from different polypeptides of different phylogenic groupings (e.g., a first component from an α and a second component from a β progenitor amino acid sequences). A chimeric polypeptide containing two or more defined segments, each of which is from a different progenitor, can be naturally-occurring or man-made (non-naturally-occurring). See Beernink PT, Granoff DM (2009) *Microbiology* 155:2873-83 for more detail on naturally-occurring chimeras. Non-naturally occurring chimeras refers to “man-made chimeras” and encompass fHbp with heterologous components that are not found in nature.

[0045] A “heterologous” or “chimeric” polypeptide may also contain two or more different components, each derived from a different fHbp (e.g. variant 1, 2, or 3). The component may be operably linked at any position along the length of the fHbp polypeptide.

[0046] “Heterologous” in the context of a polynucleotide encoding any chimeric polypeptide as described above can include operably linked nucleic acid sequence that can be derived from different genes (e.g., a first component from a nucleic acid encoding a fHBP v.1 polypeptide and a second component from a nucleic acid encoding a fHBP v.2 polypeptide) or different progenitor amino acid sequences (α or β).

[0047] Other exemplary “heterologous” nucleic acids include expression constructs in which a nucleic acid comprising a coding sequence is operably linked to a regulatory element (e.g., a promoter) that is from a genetic origin different from that of the coding sequence (e.g., to provide for expression in a host cell of interest, which may be of different genetic origin relative to the promoter, the coding sequence or both). For example, a T7 promoter operably linked to a polynucleotide encoding an fHbp polypeptide or domain thereof is said to be a heterologous nucleic acid.

[0048] “Heterologous” in the context of recombinant cells can refer to the presence of a nucleic acid (or gene product, such as a polypeptide) that is of a different genetic origin than

the host cell in which it is present. For example, a Neisserial amino acid or nucleic acid sequence of one strain is heterologous to a Neisserial host of another strain.

[0049] “Derived from” in the context of an amino acid sequence or polynucleotide sequence (e.g., an amino acid sequence “derived from” fHbp variant 3, e.g., fHbp ID 79) is meant to indicate that the polypeptide or nucleic acid has a sequence that is based on that of a reference polypeptide or nucleic acid (e.g., a naturally occurring fHbp protein or encoding nucleic acid), and is not meant to be limiting as to the source or method in which the protein or nucleic acid is made. Non-limiting examples of reference polypeptides and reference polynucleotides from which an amino acid sequence or polynucleotide sequence may be “derived from” include a naturally-occurring fHbp, e.g., fHbp ID 79, and a non-naturally-occurring fHbp. “Derived from” in the context of bacterial strains is meant to indicate that a strain was obtained through passage *in vivo*, or in *in vitro* culture, of a parental strain and/or is a recombinant cell obtained by modification of a parental strain.

[0050] “Conservative amino acid substitution” refers to a substitution of one amino acid residue for another sharing chemical and physical properties of the amino acid side chain (e.g., charge, size, hydrophobicity/hydrophilicity). “Conservative substitutions” are intended to include substitution within the following groups of amino acid residues: gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr. Guidance for such substitutions can be drawn from alignments of amino acid sequences of polypeptides presenting the epitope of interest.

[0051] The term “protective immunity” means that a vaccine or immunization schedule that is administered to a mammal induces an immune response that prevents, retards the development of, or reduces the severity of a disease that is caused by *N. meningitidis*, or diminishes or altogether eliminates the symptoms of the disease. Protective immunity can be accompanied by production of bactericidal antibodies. It should be noted that production of bactericidal antibodies against *N. meningitidis* is accepted in the field as predictive of a vaccine's protective effect in humans. (Goldschneider et al. (1969) *J. Exp. Med.* 129:1307; Borrow et al. (2001) *Infect Immun.* 69:1568).

[0052] The phrase “a disease caused by a strain of *Neisseria meningitidis*” encompasses any clinical symptom or combination of clinical symptoms that are present in an infection of a human with a *N. meningitidis*. These symptoms include but are not limited to: colonization of the upper respiratory tract (e.g., mucosa of the nasopharynx and tonsils) by a pathogenic strain of *N. meningitidis*, penetration of the bacteria into the mucosa and the submucosal

vascular bed, septicemia, septic shock, inflammation, haemorrhagic skin lesions, activation of fibrinolysis and of blood coagulation, organ dysfunction such as kidney, lung, and cardiac failure, adrenal hemorrhaging and muscular infarction, capillary leakage, edema, peripheral limb ischaemia, respiratory distress syndrome, pericarditis and meningitis.

[0053] The phrase “specifically binds to an antibody” or “specifically immunoreactive with”, in the context of an antigen (e.g., a polypeptide antigen) refers to a binding reaction which is based on and/or is probative of the presence of the antigen in a sample which may also include a heterogeneous population of other molecules. Thus, under designated conditions, the specified antibody or antibodies bind(s) to a particular antigen or antigens in a sample and do not bind in a significant amount to other molecules present in the sample.

“Specifically binds to an antibody” or “specifically immunoreactive with” in the context of an epitope of an antigen (e.g., an epitope of a polypeptide) refers to a binding reaction which is based on and/or is probative of the presence of the epitope in an antigen (e.g., polypeptide) which may also include a heterogeneous population of other epitopes, as well as a heterogeneous population of antigens. Thus, under designated conditions, the specified antibody or antibodies bind(s) to a particular epitope of an antigen and do not bind in a significant amount to other epitopes present in the antigen and/or in the sample.

[0054] The phrase “in a sufficient amount to elicit an immune response” means that there is a detectable difference between an immune response indicator measured before and after administration of a particular antigen preparation. Immune response indicators include but are not limited to: antibody titer or specificity, as detected by an assay such as enzyme-linked immunoassay (ELISA), bactericidal assay, flow cytometry, immunoprecipitation, Ouchterlony immunodiffusion; binding detection assays of, for example, spot, Western blot or antigen arrays; cytotoxicity assays, etc.

[0055] A “surface antigen” is an antigen that is present in a surface structure of *N. meningitidis* (e.g. the outer membrane, capsule, pili, etc.).

[0056] “Isolated” refers to a molecule of interest that is in an environment different from that in which the molecule may naturally occur. “Isolated” is meant to include compounds/polypeptides that are within samples that are substantially enriched for the compound/polypeptides of interest and/or in which the compound/polypeptides of interest is partially or substantially purified.

[0057] “Enriched” means that a sample is non-naturally manipulated (e.g., by a scientist or a clinician) so that a compound of interest is present in a greater concentration (e.g., at

least a three-fold greater, at least 4-fold greater, at least 8-fold greater, at least 64-fold greater, or more) than the concentration of the compound in the starting sample, such as a biological sample (e.g., a sample in which the compound naturally occurs or in which it is present after administration), or in which the compound was made (e.g., as in a bacterial polypeptide, antibody, polypeptide, and the like).

[0058] A “knock-out” or “knockout” in the context of a target gene refers to an alteration in the sequence of the gene that results in a decrease of function of the target gene, e.g., such that target gene expression is undetectable or insignificant, and/or the gene product is not functional or not significantly functional. For example, a “knockout” of a gene involved in lipopolysaccharide (LPS) synthesis indicates that function of the gene has been substantially decreased so that the expression of the gene is not detectable or is only present at insignificant levels and/or a biological activity of the gene product (e.g., an enzymatic activity) is significantly reduced relative to prior to the modification or is not detectable. “Knock-outs” encompass conditional knock-outs, where alteration of the target gene can occur upon, for example, exposure to a predefined set of conditions (e.g., temperature, osmolarity, exposure to substance that promotes target gene alteration, and the like). A “knock-in” or “knockin” of a target gene refers to a genetic alteration in a gene that that results in an increase in a function provided by the target gene.

NON-NATURALLY OCCURRING fHBP POLYPEPTIDES

[0059] Before describing further fHbps contemplated by the present disclosure, it is helpful to describe some naturally-occurring fHbps. Unique naturally-occurring fHbps found in *N. meningitidis* are each assigned a fHbp peptide ID according to neisseria.org and pubmlst.org/neisseria/fHbp websites. This convention of naming fHbps will be adopted throughout the present disclosure.

[0060] For convenience and clarity, the native amino acid sequence of fHbp ID 1 (v.1 fHbp of the *N. meningitidis* strain MC58) is selected as a reference sequence for describing amino acid position in all naturally occurring and non-naturally occurring fHbp amino acid sequences, encompassing chimeric and/or mutants of fHbps described herein. The amino acid sequence of fHbp ID 1 is presented below:

fHbp ID1 (v. 1)

CSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAQAQAEK
 TYGNGDSLNTGKLKNDKVSFRDFIRQIEVDGQLITLESQVYKQSHSALTAQTEQ
 IQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTITI
 DFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEGSYSLGIF
 GGKAQEVAGSAEVKTVNGIRHIGLAAKQ (SEQ ID NO: 1).

[0061] In referring to an amino acid residue position in a fHbp, the position number used herein corresponds to the amino acid residue number of fHbp ID 1.

[0062] See Figure 4 for an alignment of various fHbps and the amino acid residues in each fHbp corresponding to those of fHbp ID 1. As seen in Figure 4 and SEQ ID NO: 1, position number 1 refers to the first amino acid residue shown in fHbp ID 1, which is a cysteine. The fHbp referred to herein may sometimes contain an additional leader sequence at the N-terminus. For example, fHbp ID 1 may have a leader sequence of

VNRTAFCCSLTALILTA (SEQ ID NO: 2) at the N-terminus. However, amino acid position number 1 in any fHbp is still defined herein as the position that corresponds to the cysteine at amino acid position 1 shown above for fHbp ID 1 in an alignment, which amino acid is the first residue after the leader sequence, if present.

[0063] Figure 4 shows an alignment of fHbp sequences of variant 1, 2, and 3 fHbps. Note that the amino acid position 230 in fHbp ID 28 (variant 3) or fHbp ID 79 (variant 3) corresponds to amino acid position 223 of fHbp ID 1. With reference to v. 2 fHbp (e.g., fHbp ID22), the amino acid position is 222. H223 is indicated with an arrow. The alignment was performed using ClustalW available at European Bioinformatic Institute website. Amino acid identity is represented by an asterisk; strong similarity is represented by a colon; weak similarity is represented by a period; no similarity is represented by a blank space.

[0064] The present disclosure provides non-naturally occurring fHbps derived from a naturally occurring variant 3 fHbp (v. 3 fHbp), compositions comprising same, and methods of use of the fHbps non-naturally occurring and compositions. In certain embodiments, the non-naturally occurring fHbp comprises a substitution of the histidine at position 223 of the naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan, wherein the numbering of position 223 is based on the numbering of the mature fHbp ID 1, wherein the non-naturally occurring fHbp has lower affinity for human factor H (fH) than the naturally occurring variant 3 fHbp.

With reference to the sequence of fHbp ID 79 or fHbp ID 28, the histidine that is substituted in the non-naturally occurring fHbps described herein, corresponds to position

230. Specifically, the histidine that is mutated is the H within the sequence TYHLA of the amino acid sequence of v. 3 fHbp.

[0065] Human factor H (“human fH”) as used herein, refers to a protein comprising an amino acid sequence as shown below (SEQ ID NO: 3), and naturally-occurring human allelic variants thereof.

Human factor H (fH)

MRLAKIICLMLWAICVAEDCNELPPRRNTEILTGSWSDQTYPEGTQAIYKCRPGYRS
 LGNVIMVCRKGEWVALNPLRKCQKRPCGHPGDTFPGTFTLTGGNVFEYGVKAVYT
 CNEGYQLLGEINYRECDTDGWTNDIPICEVVKCLPVTAPENGKIVSSAMEPDREYHF
 GQAVRFVCNSGYKIEGDEEMHCSDDGFWSKEKPKCVEISCKSPDVINGSPISQKIIYK
 ENERFQYKCNMGYEYSERGDAVCTESGWRPLPSCEEKSCDNPYIPNGDYSPLRIKHR
 TGDEITYQCRNGFYPATRGNTAKCTSTGWIPAPRCTLKPCDYPDIKHGGLYHENMRR
 PYFPVAVGKYYSYYCDEHFETPSGSYWDHIHCTQDGWSPA VPCLRKCYFPYLENGY
 NQNHGRKFVQGKSIDVACHPGYALPKAQT TVTCMENGWSPTPRCIRVKTCSSIDI
 ENGFISESYTYALKEKAKYQCKLGYVTADGETSGSIRCGKDGWSAQPTCIKSCDIP
 VFMNARTKNDFTWFKLNDTLDYEC HDGYESNTGSTTGSIVCGYNGWSDLPICYERE
 CELPKIDVHLVPDRKKDQYKVGEVLKFSCKPGFTIVGPNSVQCYHFGLSPLPICKEQ
 VQSCGPPPELLNGNVKEKTKEEYGHSEVVEYYCNPRFLMKGPNKIQCVDGEWTTLP
 VCIVEESTCGDIPELEHGWAQLSSPPYYYGDSVEFNCSESFTMIGHRSITCIHGVWTQL
 PQCVAIDKLKKCKSSNLIILEEHLKNKKEFDHNSNIRYRCRGKEGWIHTVCINGRWD
 EVNCSMAQIQLCPPPPQIPNSHNMTTTLNYRDGEKVS VLCQENYLIQEGEEITCKDGR
 WQSIPLCVEKIPCSQPPQIEHGTINSSRSSQESYAHGTKLSYTCEGGFRISEENETTCYM
 GKWSSPPQCEGLPCKSPPEISHGVVAHMSDSYQYGEEV TYKCFEGFGIDGPAIAKCL
 GEKWSHPPSCIKTDCLSLPSFENAIPMGEKKDVYKAGEQVTYTCATYYKMDGASNV
 TCINSRWTGRPTCRDTSCVNPPTVQNAYIVSRQMSKYPSGERVRYQCRSPYEMFGDE
 EVMCLNGNWTEPPQCKDSTGKCGPPPIDNGDITSFPLSVYAPASSVEYQCQONLYQL
 EGNKRITCRNGQWSEPPKCLHPCVISREIMENYNIALRWTA KQKLYSRTGESVEFVC
 KRGYRLSSRSHTLRTTCWDGKLEYPTCAKR

[0066] Naturally occurring fHbp has a high probability to be complexed with fH, the bound fH can mask one or more epitopes on the fHbp from a host's immune system. Accordingly, fHbp that is complexed and/or bound with fH may not be as effective an immunogen as an fHbp that is not so complexed. Conversely, fHbps that have a relatively low affinity for fH, when administered as an immunogen (e.g., in a vaccine composition), can present epitopes to

the immune system of an immunized host that an fHbp that has high affinity for fH does not. The non-naturally occurring fHBps disclosed herein have a low affinity for human fH and are useful in eliciting bactericidal antibodies and/or providing protective immunity against *N. meningitidis*. A non-naturally occurring fHbp is not found in nature and is made by a human and/or intentionally modified by a human. A non-naturally occurring subject fHbp can be made via chemical synthesis or recombinant methods. A non-naturally occurring fHbp includes a mutation relative to the naturally occurring fHbp from which it was derived. As such, comprises a non-naturally occurring fHbp of the present disclosure includes a non-naturally occurring fHbp amino acid sequence.

[0067] As used herein, “low affinity”, “lower affinity”, or “low fH binder” refers to fHBps that have a binding affinity for a human fH that is lower than that of a v. 3 fHbp (e.g., fHbp ID79).

[0068] The binding affinity of the non-naturally occurring fHBps disclosed herein and human fH is 85% or less of the binding affinity of a wildtype v. 3 fHbp for human fH. For example, in some embodiments, the binding affinity of a subject non-naturally occurring fHbp for human fH is from about 85% to about 75%, from about 75% to about 65%, from about 65% to about 55%, from about 55% to about 45%, from about 45% to about 35%, from about 35% to about 25%, from about 25% to about 15%, from about 15% to about 10%, from about 10% to about 5%, from about 5% to about 2%, from about 2% to about 1%, or from about 1% to about 0.1%, or less than 0.1%, of the binding affinity of a wildtype fHbp for human fH. As an example, in some embodiments, the binding affinity of a subject non-naturally occurring fHbp for human fH is from about 85% to about 75%, from about 75% to about 65%, from about 65% to about 55%, from about 55% to about 45%, from about 45% to about 35%, from about 35% to about 25%, from about 25% to about 15%, from about 15% to about 10%, from about 10% to about 5%, from about 5% to about 2%, from about 2% to about 1%, or from about 1% to about 0.1%, or less than 0.1%, of the binding affinity of v. 3 fHbp for human fH.

[0069] Binding affinity can be described in terms of the dissociation constant (K_D). The subject non-naturally occurring fHBps and human fH can have a dissociation constant (K_D ; M) that is at least more than about 80%, at least more than about 100%, at least more than about 120%, at least more than about 140%, at least more than about 160%, at least more than about 200%, or more than K_D of wild type fHbp (such as, v. 3 fHBps, e.g., fHbp ID 79 or fHbp ID 28) and human fH. The K_D of a subject non-naturally occurring fHbp can also be

described as about 2X (2 times), about 3X, about 5X, about 10X, about 15X, about 20X, up to about 50 or more times the K_D of fHbp ID 79. For example, a subject non-naturally occurring fHbp and human fH can have a K_D that is 110% of or about 15X that of fHbp ID 79 and human fH.

[0070] As stated above, the subject non-naturally occurring fHbps are derived from a v. 3 fHbp. fHbp classified as variant group 3 (v. 3) are described in Maignani et al (2003) *J Exp Med* 197:789-99 and Pajon R et al (2010) *Vaccine* 28:2122-9. A list of known v. 3 fHbps and GenBank Accession numbers are provided in Beernink P.T. and Granoff D. M. (2009) *Microbiology*. 2009 Sep; 155(Pt 9):2873-83. Table 1 shows a list of exemplary v. 3 fHbp including the source *N. meningitidis* strain, the capsular group, and corresponding modular group, peptide ID, GenBank Accession Number.

Table 1

Source Strain	Capsular Group	Variant Group‡	fHbp SubFamily§	fHbp Peptide IDI	fHbp Modular Group*	GenBank Accession Number
CDC-1135	Y	3	A	46	II	AAR84437
M1239	B	3	A	28	II	ABF82029
03S-0451	B	3	A	76	II	ACJ04735
03S-0669	B	3	A	29	II	GQ219776
SK104	B	3	A	99	II	GQ219769
M01 240988	B	3	A	30	II	ACA52541
M06 240137	B	3	A	64	II	ACB38148
M98 250771	B	3	A	45	V	AAR84436
M97 252153	B	3	A	47	V	AAR84438
S3032	B	3	A	79	V	ACH48234
M08 240023	B	3	A	84	V	ACM44943
C2120	C	3	A	59	V	ABC59061
MD1475	B	3	A	82	V	GQ219772
M01 240355	B	3	A	31	V	ACB38139
M08 240109	B	3	A	85	V	ACM44944
M06 240138	B	3	A	70	V	ACM44938
M01 242162	B	3	A	72	V	ACM44939
MA-5756	C	3	A	67	VIII	Not Available†

19498	B	3	A	175	IX	ACI46928
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‡ Variant group defined by Massignani *et al* (2003)

§ Subfamily as defined by Fletcher *et al* (2004)

|| Peptide identification number in the fHbp peptide database at neisseria.org

* Modular group defined by different combinations of the five respective α and β segments. Group II is composed entirely of β type segments, while group V is composed of natural chimera of α and β segments.

† Sequence of fHbp ID 67 is available at Public databases for molecular typing and microbial genome diversity (at pubmlst.org) and is also provided here (see SEQ ID NO: 21).

[0071] The amino acid sequences of some examples of naturally-occurring v. 3 fHbps are shown below. Although the v. 3 fHbps include a leader sequence, the sequences shown below do not include this leader sequence and instead start at a cysteine residue which corresponds to position 1 with reference to the fHbp ID1 sequence provided above.

[0072] FHbp ID 28

CSSGGGGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLQAQG
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQNHSAV
VALQIEKINNPKDTSINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDPNG
RLHYSIDFTKKQGYGRIEHLKTLEQNVELAAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 4)

[0073] fHbp ID 46

CSSGGGGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLQAQG
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDPNR
LHYSIDFTKKQGYGGIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGT
HLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 5)

[0074] fHbp ID 76

CSSGGGGSGGIAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLQAQGA
KTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAVVA
LQIEKINNPKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDPNR
LHYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ (SEQ ID NO: 6)

[0075] fHbp ID 29

CSSGGGGSGGGGVAADIGAGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLSAQG
AEKTFKAGGKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKTDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDPNG
RLHYTIDFTNKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 7)

[0076] fHbp ID 99

CSSGGGGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLSAQG
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDPNGR
LHYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGT
HLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 8)

[0077] fHbp ID 30

CSSGGGGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLSAQG
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQNHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGDKAEYHGKAFSSDDPNGR
LHYTIDFTNKQGYGRIEHLKTPELNVDLASAELKADEKSHAVILGDTRYGSEEKGT
HLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 9)

[0078] fHbp ID 64

CSSGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLSAQGAEK
TFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQNHSAVVAL
QIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDPNGR
LHYTIDFTNKQGYGRIEHLKTPELNVDLASAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 10)

[0079] fHbp ID 45

CSSGSGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLSAQGA
EKTFKVGDKNLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAVV
ALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDAGGKL

TYTIDFAAKQGHGKIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGTYH
LALFGDRAQEIAGSATVKIREKVHEIGIAGKQ (SEQ ID NO: 11)

[0080] fHbp ID 47

CSSGGGGVAADIGAGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLAQAQGAECTF
KVGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQNHSAVVALQIE
KINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAHEYHGKAFSSDDAGGKLTYTID
FAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALF
GDRAQEIAGSATVKIGEKVHEISIAGKQ (SEQ ID NO: 12)

[0081] fHbp ID 79

CSSGGGGSGSGGVAADIGTGLADALTTPLDHKDKGLKSLTLEDSIPQNGTLTLAQQ
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAHEYHGKAFSSDDAGGK
LTYTIDFAAKQGHGKIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGTY
HLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 13)

[0082] fHbp ID 84

CSSGGGGSGGGGVAADIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLAQQ
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAHEYHGKAFSSDDAGGK
LTYTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYDSEEKGTY
HLALFGDRAQEIAGSATVKIGEKVHEISIAGKQ (SEQ ID NO: 14)

[0083] fHbp ID 59

CSSGGGGSGSGGVAADIGTGLADALTTPLDHKDKGLKSLTLEDSIPQNGTLTLAQQ
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAHEYHGKAFSSDDAGGK
LTYTIDFASKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTY
HLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 15)

[0084] fHbp ID 82

CSSGGGGVAAIDIGTGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLAQGAECTF
KAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQNHSAVVALQIE
KINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPVGKAEYHGKAFSSDDAGGKLTYTID
FAAKQGHGKIEHLKSPELNVELATAELKADEKSHAVILGDTRYGSEEKGTYHLALFG
DRAQEIAGSATVKIREKVHEIGIAGKQ (SEQ ID NO: 16)

[0085] fHbp ID 31

CSSGGGGSGSGGVAADIGTGLADALTTPLDHKDKGLKSLTLEDSIPQNGTLTLAQGA
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDAGGK
LTYTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 17)

[0086] fHbp ID 85

CSSGGGGSGSGGVAADIGTGLADALTTPLDHKDKGLKSLTLEDSIPQNGTLTLAQGA
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDAGGK
LTYTIDFAAKQGHGKIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ (SEQ ID NO: 18)

[0087] fHbp ID 70

CSSGGGGSGGGGVAADIGAGLADALTAPLDHKDKGLKSLTLEDSIPQNGTLTLAQGA
AEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDAGGK
LTYTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 19)

[0088] fHbp ID 72

CSSGGGGSGGVAADIGTGLADALTTPLDHKDKGLKSLTLEDSIPQNGTLTLAQGA
EAEKTFKAGDKDNSLNTGKLKNDKISRFDVQKIEVDGQTITLASGEFQIYKQDHSAV
VALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPGGKAEYHGKAFSSDDAGGK
LTYTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGT
YHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 20)

YTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHL
ALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 20)

[0089] fHbp ID 67

CSSGGGGSGGGGVAADIGAGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLSAQG
AERTFKAGDKDNSLNTGKLKNDKISRFDFFIRQIEVDGQLITLESGEFQIYKQDHSADV
ALQIEKINNPKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNRL
HYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYH
LALFGDRAQEIAGSATVKIREKVHEIGIAGKQ (SEQ ID NO: 21)

[0090] fHbp ID 175

CSSGGGGSGGGGVAADIGAGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLSAQG
AERTFKAGDKDNSLNTGKLKNDKISRFDFFIRQIEVDGQLITLESGEFQIYKQDHSADV
ALQIEKINNPKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKL
TYTIDFAAKQGHGKIEHLKTPEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYH
LALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 22)

[0091] As noted above, the non-naturally occurring fHbps disclosed herein may be derived from a naturally occurring v. 3 fHbp. Exemplary v. 3 fHbp are listed above. Naturally-occurring fHbps have variable segments derived from different progenitors (α and/or β). Due to the variable segments, the molecular architecture has been shown to be modular and fHbp variants can be subclassified in modular groups according to different combinations of five variable segments (A, B, C, D, and E), each derived from one of two genetic lineages, designated α - or β -types (Pajon R et al. (2010) *Vaccine* 28:2122-9; Beernink PT, Granoff DM (2009) *Microbiology* 155:2873-83). Six modular groups, designated I to VI account for >95% of all known fHbp variants (Pajon R et al. (2010) *Vaccine* 28:2122-9).

[0092] A schematic representation of fHbp modular structure is provided in Fig. 6. Schematic of nine fHbp modular groups deduced from phylogenic analysis of 242 unique amino acid variants are shown. The respective Massignani variant group designations, and the number of unique sequences observed within each fHbp modular group, are shown. Variable segments derived from α lineage are depicted in grey and those derived from β lineage are depicted in white.

[0093] In certain embodiments, the v. 3 fHbp may be a modular group II fHbp. Group II fHbps are composed entirely of β type segments (fHbp variable segments: A_β , B_β , C_β , D_β , and E_β). Exemplary modular group II fHbp are provided in Table 1.

[0094] In certain embodiments, the v. 3 fHbp may be a modular group V fHbp. Modular group V fHbps are composed of natural chimera of α and β segments and have the following fHbp variable segments from the designated progenitors: A_β , B_β , C_β , D_α , and E_β . Exemplary modular group V fHbp are provided in Table 1.

[0095] In certain embodiments, the v. 3 fHbp may be a modular group VIII fHbp. Modular group VIII fHbps are composed of α and β segments and have the following fHbp variable segments from the designated progenitors: A_β , B_α , C_β , D_β , and E_β . Exemplary modular group VIII fHbp are provided in Table 1.

[0096] In certain embodiments, the v. 3 fHbp may be a modular group IX fHbp. Modular group IX fHbps are composed of α and β segments and have the following fHbp variable segments from the designated progenitors: A_β , B_α , C_β , D_α , and E_β . Exemplary modular group IX fHbp are provided in Table 1.

[0097] In certain embodiments, the non-naturally occurring fHbp comprising a substitution of the histidine at position 223 of a naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan, may have an amino acid sequence that is at least 90% identical to the amino acid sequences of the naturally occurring variant 3 fHbp. In certain cases, the identity may be within a segment (e.g., variable segment as defined in a modular architecture), in two or more variable segments, or in the full-length mature protein.

[0098] The subject non-naturally occurring fHbp can comprise an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, amino acid sequence identity with a variant 3 fHbp (for example, fHbp 79). In certain cases, the subject non-naturally occurring fHbp may differ from the amino acid sequence of the v. 3 fHbp by from 1 amino acid (aa) to 25 amino acids, e.g., differs from the amino acid sequence of the v. 3 fHbp by 1 aa, 2 aa, 3 aa, 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 13 aa, 15 aa, 18 aa, 20 aa, 22 aa, 24 aa, or 25 aa. Thus, e.g., a subject non-naturally occurring fHbp can have at most one, at most two, at most three, at most four, at most six, at most eight, at most ten, at most twelve, at most fourteen, at most sixteen, at most eighteen, up to at most 25 or more modifications (e.g. substitutions, deletions, or insertions) relative to a v. 3 fHbp from which the subject fHbp is

derived. The one or more amino acid alterations can decrease the affinity of the fHbp for human fH relative to a fHbp that is not altered.

[0099] In some embodiments, a subject non-naturally occurring fHbp comprises a substitution of the histidine at position 223 of a naturally occurring variant 3 fHbp with arginine. As noted above, the v. 3 fHbp may be fHbp ID 46, fHbp ID 28, fHbp ID 76, fHbp ID 29, fHbp ID 99, fHbp ID 30, fHbp ID 64, fHbp ID 45, fHbp ID 47, fHbp ID 79, fHbp ID 84, fHbp ID 59, fHbp ID 82, fHbp ID 31, fHbp ID 85, fHbp ID 70, fHbp ID 72, fHbp ID 67, or fHbp ID 175.

[00100] An alignment of exemplary v. 3 fHbps is provided in Figure 5. The position of H223 is also indicated. The reference sequence, fHbp ID 1, is also included in the alignment. Notably, fHbp ID 1 does not have a histidine at position 223. The alignment was performed using ClustalW available at European Bioinformatic Institute website. Amino acid identity is represented by an asterisk; strong similarity is represented by a colon; weak similarity is represented by a period; no similarity is represented by a blank space.

[00101] As shown herein, a fHbp derived from v. 3 fHbp and that contains H223R substitution has decreased binding to human fH relative to the naturally-occurring v. 3 fHbp. Thus, non-naturally fHbp containing the amino acid substitution that is conservative relative to the arginine substitution are also expected to have decreased binding to human fH compared to the naturally-occurring v. 3 fHbp. As such, the present disclosure contemplates conservative amino acid substitutions relative to arginine (R), such as the amino acid substitutions H223K is also contemplated. Additionally, an amino acid substitution that provides steric hinderance to the binding of fH to fHbp are also contemplated-exemplary amino acid substitutions include substitution with an amino acid having a bulky hydrophobic side chain, such as phenylalanine, tyrosine, and tryptophan. As such, the amino acid substitutions H223F, H223Y, and H223W are also contemplated.

[00102] In general, the subject non-naturally occurring fHbp does not include a substitution of the histidine at position 223 with alanine.

[00103] One feature of a subject non-naturally occurring fHbp is that when administered to a host (e.g., mammals such as mice or human), the subject fHbp can elicit a bactericidal response at a level comparable or higher than the bactericidal response elicited by the v. 3 fHbp from which it was derived (e.g. fHbp ID 79, 46, 28, 67, 175, or 59). Methods for determining levels of bactericidal response are known in the art. In certain cases, the geometric mean bactericidal titers of mice immunized with the subject fHbp is at least about

70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 110%, at least about 120%, at least about 150%, at least about 175%, at least about 200%, or more than 200%, of the geometric mean bactericidal titers of mice immunized with a variant 3 fHbp (e.g., fHbp ID 79). In some instances, the geometric mean bactericidal titer of a mouse immunized with a subject fHbp is at least 2-fold, at least 2.5-fold, at least 5-fold, at least 10-fold, or more than 10-fold, higher than the geometric mean bactericidal titer of a control mouse immunized with a variant 3 fHbp (e.g., fHbp ID 79). The subject fHbps can exclude those that elicit a bactericidal response significantly lower than that elicited by a variant 3 fHbp (e.g., fHbp ID 79).

[00104] In many cases, a subject non-naturally occurring fHbp maintains and presents a conformational epitope bound by antibodies that have bactericidal activity toward one or more *Neisseria meningitidis* strains. Thus, such fHbp mutants may maintain an epitope found in a naturally-occurring v. 3 fHbp, while exhibiting reduced binding to fH compared to the binding affinity for fH of the naturally-occurring v. 3 fHbp. Mutants that have minimal or no effect on the conformation of fHbp such that the mutant fHbp elicits bactericidal antibodies are considered good vaccine candidates. Whether a mutant has an effect on the conformation of fHbp can be determined in various ways, including binding of JAR 31, JAR 33, or JAR 11 antibodies. Accordingly, a non-naturally occurring fHbp disclosed herein may bind to JAR 31, JAR 33, or JAR 11 antibodies.

[00105] The fHbps of the present disclosure may have additional features, described in more detail below.

Chimeric fHbps

[00106] A non-naturally occurring fHbp of the present disclosure may be a chimeric fHbp. A chimeric fHbp of the present disclosure may be described as having N-terminal domain (fHbpN) of fHbp from a variant group 1 or 2 (v. 1 or v. 2 fHbp) while the C-terminal domain (fHbpC) may be derived from a v. 3 fHbp (e.g. fHbp ID 79), the non-naturally occurring fHbp comprising a substitution of the histidine at position 223 of the naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan.

[00107] In certain embodiments, the chimeric non-naturally occurring fHbp of the present disclosure may have a fHbpN domain of fHbp from a variant group 1 (e.g., fHbp ID 1) and fHbpC domain derived from a v. 3 fHbp (e.g. fHbp ID 28), the chimeric non-naturally

occurring fHbp comprising a substitution of the histidine at position 223 of the naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan.

[00108] In certain embodiments, the chimeric non-naturally occurring fHbp of the present disclosure may have a fHbpN domain derived from a variant group 1 fHbp (e.g., fHbp ID 1) and fHbpC domain derived from a v. 3 fHbp (e.g. fHbp ID 79), the non-naturally occurring fHbp comprising a substitution of the histidine at position 223 of the naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan. In certain cases, the fHbpN domain may have a substitution of the arginine at position 41 of a naturally occurring v. 1 fHbp. In certain cases, the substitution may be R41S.

[00109] “fHbpN” refers to a contiguous amino acid sequence that starts at about residue position 8 and ends at about residue position 136. “fHbpC” refers to a contiguous amino acid sequence that starts at about residue position 141 and ends at about residue position 255. Intervening sequence between fHbpN and fHbpC is a linker between the two domains.

[00110] Exemplary chimeric fHbp that may be modified to include the H223R, H223W, H223F, H223Y, or H223K substitution may be any known man-made chimera, such as those described in Beernink et al. (2008) *Infect. Immun.* 76:2568-2575 and WO 2009/114485, disclosure of which is incorporated herein by reference. The chimera containing the substitution may have a decreased affinity for human fH relative to the corresponding chimeric fHbp, while still maintaining epitopes important for eliciting bactericidal response, such as those found in the corresponding chimeric fHbp. fHbp epitopes that may be maintained in the modified chimeric includes those that are found in the corresponding chimeric fHbp such as those described in WO 2009/114485, disclosure of which is incorporated herein by reference. For example, a modified chimeric fHbp can contain epitopes important for eliciting bactericidal antibody response against strains containing variant 1 fHbp (e.g. epitopes in the N-terminal domain such as those defined by mAb JAR 4 and/or JAR 5) and/or against strains containing variant 2 and/or 3 fHbp (e.g. epitopes defined by mAb JAR 10, JAR 11, JAR 13, and/or JAR 36).

[00111] Exemplary Chimera are shown below:

[00112] Chimera 1/79

[00113] cssggggvaadigagladaltapldhkdkgqlsltdqsvrkneklklaaqaektygngdsIntgklkndkvsrfd
irqievdgqlitlesgefvykqshsaltafqteqiqdsehsgkmvakraqfrigdiaGEHTAFNQLPGGKAEYHGK

AFSSDDAGGKLTYTIDFAAKQGHGKIEHLKTPEQNVELASAELKADEKSHAVILGDT RYGSEEKGTY**HL**LALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 23). The lower case letters correspond to the amino acid sequence that is derived from fHbp ID 1 while the upper case letters correspond to the amino acid that is derived from fHbp ID 79. Position corresponding to H223 in fHbp ID 79 is the bolded and underlined. Accordingly, the chimeric non-naturally occurring fHbp of the present disclosure may include the sequence of Chimera 1/79 with a substitution of H223 into H223R, H223W, H223F, H223Y, or H223K.

[00114] Chimera 1(R41S)/79

[00115] cssggggvaadigagladaltapldhkdkglsitldqsv**sk**neklklaaqgaektygngdsIntgklkndkvsrfdfrqievdgqlitlesgefvykqshsalfqteqiqdsehsgkmvkrqfrigdiaGEHTAFNQLPGGKAEYHGK AFSSDDAGGKLTYTIDFAAKQGHGKIEHLKTPEQNVELASAELKADEKSHAVILGDT RYGSEEKGTY**HL**LALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ (SEQ ID NO: 24). The lower case letters correspond to the amino acid sequence that is derived from fHbp ID 1 while the upper case letters correspond to the amino acid that is derived from fHbp ID 79. Position 41 includes a substitution of the R with a serine. Position corresponding to H223 in fHbp ID 79 is the bolded and underlined. Accordingly, the chimeric non-naturally occurring fHbp of the present disclosure may include the sequence of Chimera 1(R41S)/79 with a substitution of H223 into H223R, H223W, H223F, H223Y, or H223K.

[00116] In general, the subject non-naturally occurring fHbp does not include a substitution of the histidine at position 223 with alanine.

[00117] The subject non-naturally occurring chimeric fHbp can comprise an amino acid sequence having at least 90%, at least 95%, at least 98%, at least 99%, amino acid sequence identity with a variant 3 fHbp (for example, fHbp 79). The identity may be within the region of the subject chimeric fHbp derived from v. 3 fHbp and the corresponding region of the v. 3 fHbp. For example, in a chimeric protein where the fHbpC domain is derived from a v. 3 fHbp, the identity may be in the fHbp C domains.

[00118] One feature of a subject chimeric non-naturally occurring fHbp is that when administered to a host (e.g. mammals such as mice or human), the subject fHbp can elicit a bactericidal response at a level comparable or higher than the bactericidal response elicited by the variant group fHbps from which the chimera is derived (e.g. fHbp ID 1 and fHbp ID 79).

[00119] Methods for determining levels of bactericidal response are known in the art. In certain cases, the geometric mean bactericidal titers of mice immunized with the subject

chimeric fHbp is at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 110%, at least about 120%, at least about 150%, at least about 175%, at least about 200%, or more than 200%, of the geometric mean bactericidal titers of mice immunized with fHbp ID 1 or fHbp ID 79. In some instances, the geometric mean bactericidal titer of a mouse immunized with a subject fHbp is at least 2-fold, at least 2.5-fold, at least 5-fold, at least 10-fold, or more than 10-fold, higher than the geometric mean bactericidal titer of a control mouse immunized with fHbp ID 1 or fHbp ID 79.

[00120] The subject fHbps can exclude those that elicit a bactericidal response significantly lower than that elicited by fHbp ID 1 or fHbp ID 79.

[00121] In many cases, a subject non-naturally occurring chimeric fHbp maintains and presents a conformational epitope bound by bactericidal antibodies that have bactericidal activity toward one or more *Neisseria meningitidis* strains. Thus, such fHbp mutants may maintain an epitope found in a naturally-occurring fHbp, while exhibiting reduced binding to fH compared to the binding affinity for fH of a naturally-occurring fHbp. Non-naturally occurring chimeric fHbp of the present disclosure that have have minimal or no effect on the conformation of fHbp such that the chimeric fHbp elicits bactericidal antibodies are considered good vaccine candidates. Whether a variant has an effect on the conformation of fHbp can be determined in various ways, including binding of antibodies, such as, JAR 4, JAR 5, JAR 31, JAR 33, or JAR 11.

[00122] The fHbps of the present disclosure may have additional features, described in more detail below.

Conjugates

[00123] The subject fHbps of the present disclosure may contain one or more additional elements at the N- and/or C-terminus of the polypeptide, such as a polypeptide (e.g. having an amino acid sequence heterologous to the subject fHbp) and/or a carrier molecule. The additional heterologous amino acid sequences may be fused, e.g., to provide an N-terminal methionine or derivative thereof (e.g., pyroglutamate) as a result of expression in a bacterial host cell (e.g., *E. coli*) and/or to provide a chimeric polypeptide having a fusion partner at its N-terminus or C-terminus. Fusion partners of interest include, for example, glutathione-S-transferase (GST), maltose binding protein (MBP), His₆-tag, and the like, as well as leader peptides from other proteins, particularly lipoproteins. Fusion partners can provide for

additional features, such as in facilitating isolation, purification, detection, immunogenicity of the subject fHbp.

[00124] Other elements that may be linked to the subject fHbp include a carrier molecule (e.g., a carrier protein, e.g. keyhole limpet hemocyanin (KLH)). Additional elements may be linked to the peptide via a linker, e.g. a flexible linker. Carriers encompass immunomodulators, a molecule that directly or indirectly modifies an immune response. A specific class of immunomodulators includes those that stimulate or aid in the stimulation of an immunological response. Examples include antigens and antigen carriers such as a toxin or derivative thereof, including tetanus toxoid. Other carrier molecules that facilitate administration and/or to increase the immunogenicity in a subject to be vaccinated or treated against *N. meningitidis* are also contemplated. Carrier molecules can also facilitate delivery to a cell or tissue of interest. The additional moiety may also aid in immunogenicity or forming a complex with a component in a vaccine. The carrier molecules may act as a scaffold protein to facilitate display of the epitopes on a membrane surface (e.g. a vesicle vaccine).

[00125] In one example, the subject fHbps are modified at the N- and/or C-terminus to include a fatty acid (e.g. aliphatic carboxylic acid group). The fatty acid may be covalently linked to the fHbp via a flexible linker. An example of a fatty acid that may be used to modify an end (e.g. N-terminal end, e.g., at the N-terminus) of the subject fHbp is lauric acid. Lauric acid when covalently attached to another molecule is referred to as a lauroyl group (e.g. lauroyl sulfate). Lauric acid contains twelve carbon atoms with ten methylene groups and the formula $\text{CH}_3-(\text{CH}_2)_{10}-\text{COOH}$. Other fatty acids that may be linked to the subject peptides include caprylic acid (10 C), myristic acid (14 C), and palmitic acid (16 C). For details, see Westerink MA et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:4021-4025. It is also contemplated that any hydrophobic moiety that can serve to anchor the subject fHbp into the bacterial outer membrane is contemplated herein for conjugation to a N- and/or C-terminal end (e.g., at the N-terminus) of the fHbps of the present disclosure, where the hydrophobic moiety can be optionally conjugated to the peptide through a linker, e.g., a flexible linker, as described herein. For example, a hydrophobic pentapeptide FLLAV (SEQ ID NO: 25), as described in Lowell GH et al. (1988) *J. Exp. Med.* 167:658-63.

[00126] As noted above, one way in which the fatty acid, as well as other additional elements described above, is connected to the fHbp is via a linker (e.g. lauroyl-Gly-Gly). Linkers suitable for use in modifying the fHbp of the present disclosure include “flexible linkers”. Suitable linkers can be readily selected and can be of any of a suitable of different

lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

[00127] Examples of flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, GSGGS_n and GGGS_n, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are of interest since both of these amino acids are relatively unstructured, and therefore may serve as a neutral tether between components. Glycine polymers are of particular interest since glycine accesses significantly more Ramachandran (or phi-psi) space than even alanine, and are much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11173-142 (1992)). Exemplary flexible linkers include, but are not limited GSGG (SEQ ID NO: 26), GSGGG (SEQ ID NO: 2), GSGSG (SEQ ID NO: 28), GSGGG (SEQ ID NO: 29), GGGSG (SEQ ID NO: 30), GSSSG (SEQ ID NO: 31), and the like. The ordinarily skilled artisan will recognize that design of a fHbp conjugated to any elements described above can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

[00128] Native fHbp usually contains an N-terminal cysteine to which a lipid moiety can be covalently attached. This cysteine residue is usually lipidated in the naturally-occurring protein, and can be lipidated in the subject fHBps disclosed herein. Thus, in the amino acid sequences described herein, reference to “cysteine” or “C” at this position specifically includes reference to both an unmodified cysteine as well as to a cysteine that is lipidated (e.g., due to post-translational modification). Thus, the subject fHbp can be lipidated or non-lipidated. Methods for production of lipidated proteins *in vitro*, (see, e.g., Andersson et al. (2001) *J. Immunological Methods* 255:135-48) or *in vivo* are known in the art. For example, lipidated fHbp previously has been purified from the membrane fraction of *E. coli* protein by detergent extraction (Fletcher et al. (2004) *Infection and Immunity* 72:2088-100), which method may be adapted for the production of lipidated fHbp. Lipidated proteins may be of interest as such can be more immunogenic than soluble protein (see, e.g., Fletcher et al. (2004) *Infection and Immunity* 72:2088-100).

[00129] It will be appreciated that the nucleotide sequences encoding heterologous fHBps can be modified so as to optimize the codon usage to facilitate expression in a host cell of

interest (e.g., *E. coli*, *N. meningitidis*, human (as in the case of a DNA-based vaccine), and the like). Methods for production of codon optimized sequences are known in the art.

Nucleic acids encoding fHbp

[00130] The present disclosure provides a nucleic acid encoding a subject fHbp. A subject nucleic acid will in some embodiments be present in a recombinant expression construct. Also provided are genetically modified host cells comprising a subject nucleic acid.

[00131] fHbp polypeptides, and encoding nucleic acids of the present disclosure can be derived from any suitable *N. meningitidis* strain. As is known in the art, *N. meningitidis* strains are divided into serologic groups (capsular groups), serotypes (PorB phenotypes) and subtypes (PorA phenotypes) on the basis of reactions with polyclonal (Frasch, C. E. and Chapman, 1973, *J. Infect. Dis.* 127: 149-154) or monoclonal antibodies that interact with different surface antigens. Capsular grouping traditionally has been based on immunologically detectable variations in the capsular polysaccharide but is being replaced by PCR of genes encoding specific enzymes responsible for the biosynthesis of the structurally different capsular polysaccharides. About 12 capsular groups (including A, B, C, X, Y, Z, 29-E, and W-135) are known. Strains of the capsular groups A, B, C, Y and W-135 account for nearly all meningococcal disease. Serotyping traditionally has been based on monoclonal antibody defined antigenic differences in an outer membrane protein called Porin B (PorB). Antibodies defining about 21 serotypes are currently known (Sacchi et al., 1998, *Clin. Diag. Lab. Immunol.* 5:348). Serosubtyping has been based on antibody defined antigenic variations on an outer membrane protein called Porin A (PorA). Both serotyping and serosubtyping are being replaced by PCR and/or DNA sequencing for identification of genes encoding the variable regions of PorB and PorA, respectively that are associated with mAb reactivity (e.g. Sacchi, Lemos et al., supra; Urwin et al., 1998, *Epidem. and Infect.* 120:257).

[00132] While *N. meningitidis* strains of any capsular group may be used, *N. meningitidis* strains of capsular group B can be sources from which nucleic acid encoding fHbp and domains thereof are derived.

[00133] Nucleic acids encoding fHbp polypeptides for use in construction of the subject fHbps contemplated herein are known in the art. Various fHbp and their sequences are available at neisseria.org and pubmlst.org/neisseria/fHbp websites. Examples of fHbp polypeptides are also described in, for example, US patent application number 61/174,424, PCT application number PCT/US09/36577, WO 2004/048404; Massignani et al. (2003) *J Exp Med* 197:789-799; Fletcher et al. (2004) *Infect Immun* 72:2088-2100; Welsch et al. J

Immunol 2004 172:5606-5615; and WO 99/57280. Nucleic acid (and amino acid) sequences for fHbp variants are also provided in GenBank.

[00134] For purposes of identifying relevant amino acid sequences contemplated for use in the subject fHBps disclosed herein, it should be noted that the immature fHbp includes a leader sequence of about 19 residues. Furthermore, when provided an amino acid sequence the ordinarily skilled person can readily envision the sequences of nucleic that can encode for, and provide for expression of, a polypeptide having such an amino acid sequence.

[00135] In addition to the specific amino acid sequences and nucleic acid sequences provided herein, the disclosure also contemplates polypeptides and nucleic acids having sequences that are at least 80%, at least 85%, at least 90%, or at least 95% identical in sequence to such examples of amino acid and nucleic acids. The terms “identical” or percent “identity,” in the context of two or more polynucleotide sequences, or two or more amino acid sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (e.g., at least 80%, at least 85%, at least 90%, or at least 95% identical over a specified region), when compared and aligned for maximum correspondence over a designated region, e.g., a V_E or a region of at least about 40, 45, 50, 55, 60, 65 or more amino acids or nucleotides in length, and can be up to the full-length of the reference amino acid or nucleotide sequence (e.g., a full-length fHbp). The disclosure specifically contemplates both naturally-occurring polymorphisms and synthetically produced amino acid sequences and their encoding nucleic acids.

[00136] For sequence comparison, typically one sequence acts as a reference sequence (e.g., a naturally-occurring fHbp polypeptide sequence or a segment thereof), to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer program, sequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00137] Examples of algorithms that are suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1977) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the

National Center for Biotechnology Information. Further exemplary algorithms include ClustalW (Higgins D., et al. (1994) *Nucleic Acids Res* 22: 4673-4680).

[00138] Some residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having acidic side chains is aspartate and glutamate; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine.

[00139] Sequence identity between two nucleic acids can also be described in terms of hybridization of two molecules to each other under stringent conditions. The hybridization conditions are selected following standard methods in the art (see, for example, Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). An example of stringent hybridization conditions is hybridization at 50°C or higher and $0.1 \times$ SSC (15 mM sodium chloride/1.5 mM sodium citrate). Another example of stringent hybridization conditions is overnight incubation at 42 °C in a solution: 50 % formamide, $5 \times$ SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH7.6), $5 \times$ Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in $0.1 \times$ SSC at about 65 °C. Stringent hybridization conditions are hybridization conditions that are at least as stringent as the above representative conditions, where conditions are considered to be at least as stringent if they are at least about 80% as stringent, typically at least 90% as stringent as the above specific stringent conditions.

METHODS OF PRODUCTION

[00140] The fHbps of the present disclosure can be produced by any suitable method, including recombinant and non-recombinant methods (e.g., chemical synthesis). Where the subject fHbp is produced using recombinant techniques, the methods can involve any suitable construct and any suitable host cell, which can be a prokaryotic or eukaryotic cell, usually a

bacterial or yeast host cell, more usually a bacterial cell. Methods for introduction of genetic material into host cells include, for example, transformation, electroporation, conjugation, calcium phosphate methods and the like. The method for transfer can be selected so as to provide for stable expression of the introduced fHbp-encoding nucleic acid. The fHbp-encoding nucleic acid can be provided as an inheritable episomal element (e.g., plasmid) or can be genomically integrated.

[00141] Suitable vectors for transferring fHbp-encoding nucleic acid can vary in composition. Integrative vectors can be conditionally replicative or suicide plasmids, bacteriophages, and the like. The constructs can include various elements, including for example, promoters, selectable genetic markers (e.g., genes conferring resistance to antibiotics (for instance kanamycin, erythromycin, chloramphenicol, or gentamycin)), origin of replication (to promote replication in a host cell, e.g., a bacterial host cell), and the like. The choice of vector will depend upon a variety of factors such as the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially.

[00142] In one example, the vector is an expression vector based on episomal plasmids containing selectable drug resistance markers and elements that provide for autonomous replication in different host cells (e.g., in both *E. coli* and *N. meningitidis*). One example of such a “shuttle vector” is the plasmid pFP10 (Pagotto et al. (2000) *Gene* 244:13-19).

[00143] Constructs can be prepared by, for example, inserting a polynucleotide of interest into a construct backbone, typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence can be inserted by homologous recombination or site-specific recombination. Typically homologous recombination is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence, while site-specific recombination can be accomplished through use of sequences that facilitate site-specific recombination (e.g., cre-lox, att sites, etc.). Nucleic acid containing such sequences can be added by, for example, ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence.

[00144] Vectors can provide for extrachromosomal maintenance in a host cell or can provide for integration into the host cell genome. Vectors are amply described in numerous publications well known to those in the art, including, e.g., Short Protocols in Molecular Biology, (1999) F. Ausubel, et al., eds., Wiley & Sons. Vectors may provide for expression of the nucleic acids encoding the subject fHbp, may provide for propagating the subject nucleic acids, or both.

[00145] Examples of vectors that may be used include but are not limited to those derived from recombinant bacteriophage DNA, plasmid DNA or cosmid DNA. For example, plasmid vectors such as pBR322, pUC 19/18, pUC 118, 119 and the M13 mp series of vectors may be used. pET21 is also an expression vector that may be used. Bacteriophage vectors may include λ gt10, λ gt11, λ gt18-23, λ ZAP/R and the EMBL series of bacteriophage vectors. Further vectors that may be utilized include, but are not limited to, pJB8, pCV 103, pCV 107, pCV 108, pTM, pMCS, pNNL, pHSG274, COS202, COS203, pWE15, pWE16 and the charomid 9 series of vectors.

[00146] For expression of a subject fHbp, an expression cassette may be employed. Thus, the present disclosure provides a recombinant expression vector comprising a subject nucleic acid. The expression vector provides transcriptional and translational regulatory sequences, and may provide for inducible or constitutive expression, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region. These control regions may be native to an fHbp from which the subject fHbp is derived, or may be derived from exogenous sources. In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. Promoters can be either constitutive or inducible, and can be a strong constitutive promoter (e.g., T7, and the like).

[00147] Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding proteins of interest. A selectable marker operative in the expression host may be present to facilitate selection of cells containing the vector. In addition, the expression construct may include additional elements. For example, the expression vector may have one or two replication systems, thus allowing it to be maintained in organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. In addition the

expression construct may contain a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

[00148] It should be noted that fHbps of the present disclosure may comprise additional elements, such as a detectable label, e.g., a radioactive label, a fluorescent label, a biotin label, an immunologically detectable label (e.g., an HA tag, a poly-Histidine tag) and the like. Additional elements of fHbp can be provided to facilitate isolation (e.g., biotin tag, immunologically detectable tag) through various methods (e.g., affinity capture, etc.). The subject fHbp can optionally be immobilized on a support through covalent or non-covalent attachment.

[00149] Isolation and purification of fHbp can be accomplished according to methods known in the art. For example, fHbp can be isolated from a lysate of cells genetically modified to express a fHbp, or from a synthetic reaction mix, by immunoaffinity purification, which generally involves contacting the sample with an anti-fHbp antibody (e.g., an anti-fHbp mAb, such as a JAR 5 MAb or other appropriate JAR MAb known in the art), washing to remove non-specifically bound material, and eluting specifically bound fHbp. Isolated fHbp can be further purified by dialysis and other methods normally employed in protein purification methods. In one example, the fHbp can be isolated using metal chelate chromatography methods.

Host cells

[00150] Any of a number of suitable host cells can be used in the production of fHbp. In general, the fHbp described herein may be expressed in prokaryotes or eukaryotes, usually bacteria, more usually *E. coli* or *Neisseria* (e.g., *N. meningitidis*) in accordance with conventional techniques. Thus, the present disclosure further provides a genetically modified host cell, which contains a nucleic acid encoding a subject fHbp. Host cells for production (including large scale production) of a subject fHbp can be selected from any of a variety of available host cells. Examples of host cells for expression include those of a prokaryotic or eukaryotic unicellular organism, such as bacteria (e.g., *Escherichia coli* strains), yeast (e.g., *S. cerevisiae*, *Pichia* spp., and the like), and may include host cells originally derived from a higher organism such as insects, vertebrates, particularly mammals, (e.g. CHO, HEK, and the like). Generally bacterial host cells and yeast are of particular interest for subject fHbp production.

[00151] Subject fHbps can be prepared in substantially pure or substantially isolated form (i.e., substantially free from other Neisserial or host cell polypeptides) or substantially isolated form. The subject fHbp can be present in a composition that is enriched for the polypeptide relative to other components that may be present (e.g., other polypeptides or other host cell components). Purified subject fHbp can be provided such that the polypeptide is present in a composition that is substantially free of other expressed polypeptides, e.g., less than 90%, usually less than 60% and more usually less than 50% of the composition is made up of other expressed polypeptides.

Host cells for vesicle production

[00152] Where a subject fHbp is to be provided in a membrane vesicle (as discussed in more detail below), a Neisserial host cell is genetically modified to express a subject fHbp. Any of a variety of *Neisseria* spp. strains can be modified to produce a subject fHbp, and, optionally, which produce or can be modified to produce other antigens of interest, such as PorA, can be used in the methods disclosed herein.

[00153] Methods and vectors to provide for genetic modification of Neisserial strains and expression of a desired polypeptide are known in the art. Examples of vectors and methods can be found in WO 02/09746 and O'Dwyer et al. (2004) *Infect Immun* 72:6511-80. Strong promoters, particularly constitutive strong promoters are of particular interest. Examples of promoters include the promoters of *porA*, *porB*, *lbpB*, *tbpB*, *p110*, *hpuAB*, *lgtF*, *opa*, *p110*, *lst*, *hpuAB*, and *rmp*. In certain embodiments, the Neisserial strain is modified by recombinant techniques to provide for a sufficiently high level of production of the non-naturally occurring fHbp disclosed herein. Such modified strains generally are produced so as to provide for an expression level of the non-naturally occurring fHbp that is 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 -fold or greater over fHbp production in the parental cell not genetically modified to express the non-naturally occurring fHbp. In certain embodiments, the parental cell may be genetically modified to knock out the endogenous fHbp and to overexpress the non-naturally occurring fHbp.

[00154] Pathogenic *Neisseria* spp. or strains derived from pathogenic *Neisseria* spp., particularly strains pathogenic for humans or derived from strains pathogenic or commensal for humans, are of particular interest for use in membrane vesicle production. Examples of Neisserial spp. include *N. meningitidis*, *N. flavescens*, *N. gonorrhoeae*, *N. lactamica*, *N. polysaccharea*, *N. cinerea*, *N. mucosa*, *N. subflava*, *N. sicca*, *N. elongata*, and the like.

[00155] *N. meningitidis* strains are of particular interest for genetic modification to express the subject fHbps and for use in vesicle production. The strain used for vesicle production can be selected according to a number of different characteristics that may be desired. For example, the strain may be selected according to: a desired PorA type (a “serosubtype”, as described above), capsular group, serotype, and the like; decreased capsular polysaccharide production; and the like. For example, the production strain can produce any desired PorA polypeptide, and may express one or more PorA polypeptides (either naturally or due to genetic engineering). Examples of strains include those that produce a PorA polypeptide which confers a serosubtype of P1.7,16; P1.19,15; P1.7,1; P1.5,2; P1.22a,14; P1.14 ; P1.5,10; P1.7,4; P1.12,13; as well as variants of such PorA polypeptides which may or may not retain reactivity with conventional serologic reagents used in serosubtyping. Also of interest are PorA polypeptides characterized according to PorA variable region (VR) typing (see, e.g., Russell et al. (2004) *Emerging Infect Dis* 10:674-678; Sacchi CT et al. (1998) *Clin Diagn Lab Immunol* 5:845-55; Sacchi et al (2000) *J. Infect Dis* 182:1169-1176). A substantial number of distinct VR types have been identified, which can be classified into VR1 and VR2 family “prototypes”. A web-accessible database describing this nomenclature and its relationship to previous typing schemes is found at neisseria.org/nm/typing/pora. Alignments of certain PorA VR1 and VR2 types are provided in Russell et al. (2004) *Emerging Infect Dis* 10:674-678.

[00156] Alternatively or in addition, the production strain can be a capsule deficient strain. Capsule deficient strains can provide vesicle-based vaccines that provide for a reduced risk of eliciting a significant autoantibody response in a subject to whom the vaccine is administered (e.g., due to production of antibodies that cross-react with sialic acid on host cell surfaces). “Capsule deficient” or “deficient in capsular polysaccharide” as used herein refers to a level of capsular polysaccharide on the bacterial surface that is lower than that of a naturally-occurring strain or, where the strain is genetically modified, is lower than that of a parental strain from which the capsule deficient strain is derived. A capsule deficient strain includes strains that are decreased in surface capsular polysaccharide production by at least 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85%, 90% or more, and includes strains in which capsular polysaccharide is not detectable on the bacterial surface (e.g., by whole cell enzyme-linked immunosorbent assay (ELISA) using an anti-capsular polysaccharide antibody).

[00157] Capsule deficient strains include those that are capsule deficient due to a naturally-occurring or recombinantly-generated genetic modification. Naturally-occurring capsule

deficient strains (see, e.g., Dolan-Livengood et al. (2003) *J. Infect. Dis.* 187:1616-28), as well as methods of identifying and/or generating capsule-deficient strains (see, e.g., Fisseha et al. (2005) *Infect. Immun.* 73:4070-4080; Stephens et al. (1991) *Infect Immun* 59:4097-102; Frosch et al. (1990) *Mol Microbiol.* 4:1215-1218) are known in the art.

[00158] Modification of a Neisserial host cell to provide for decreased production of capsular polysaccharide may include modification of one or more genes involved in capsule synthesis, where the modification provides for, for example, decreased levels of capsular polysaccharide relative to a parent cell prior to modification. Such genetic modifications can include changes in nucleotide and/or amino acid sequences in one or more capsule biosynthesis genes rendering the strain capsule deficient (e.g., due to one or more insertions, deletions, substitutions, and the like in one or more capsule biosynthesis genes). Capsule deficient strains can lack or be non-functional for one or more capsule genes.

[00159] Of particular interest are strains that are deficient in sialic acid biosynthesis. Such strains can provide for production of vesicles that have reduced risk of eliciting anti-sialic acid antibodies that cross-react with human sialic acid antigens, and can further provide for improved manufacturing safety. Strains having a defect in sialic acid biosynthesis (due to either a naturally occurring modification or an engineered modification) can be defective in any of a number of different genes in the sialic acid biosynthetic pathway. Of particular interest are strains that are defective in a gene product encoded by the N-acetylglucosamine-6-phosphate 2-epimerase gene (known as synX AAF40537.1 or siaA AAA20475), with strains having this gene inactivated being of especial interest. For example, in one embodiment, a capsule deficient strain is generated by disrupting production of a functional synX gene product (see, e.g., Swartley et al. (1994) *J Bacteriol.* 176:1530-4).

[00160] Capsule-deficient strains can also be generated from naturally-occurring strains using non-recombinant techniques, e.g., by use of bactericidal anti-capsular antibodies to select for strains with reduced levels of capsular polysaccharide.

[00161] Where the disclosure involves use of two or more strains (e.g., to produce antigenic compositions containing a subject fHbp-presenting vesicles from different strains), the strains can be selected so as to differ in one or more strain characteristics, e.g., to provide for vesicles that differ in the subject fHbp used, PorA, and the like.

Preparation of Vesicles

[00162] The antigenic compositions contemplated by the present disclosure generally include vesicles prepared from Neisserial cells that express a subject fHbp. As referred to herein “vesicles” is meant to encompass outer membrane vesicles as well as microvesicles (which are also referred to as blebs).

[00163] The antigenic composition can contain outer membrane vesicles (OMV) prepared from the outer membrane of a cultured strain of *Neisseria meningitidis* spp. genetically modified to express a subject fHbp. OMVs may be obtained from *Neisseria meningitidis* grown in broth or solid medium culture, preferably by separating the bacterial cells from the culture medium (e.g. by filtration or by a low-speed centrifugation that pellets the cells, or the like), lysing the cells (e.g. by addition of detergent, osmotic shock, sonication, cavitation, homogenization, or the like) and separating an outer membrane fraction from cytoplasmic molecules (e.g. by filtration; or by differential precipitation or aggregation of outer membranes and/or outer membrane vesicles, or by affinity separation methods using ligands that specifically recognize outer membrane molecules; or by a high-speed centrifugation that pellets outer membranes and/or outer membrane vesicles, or the like); outer membrane fractions may be used to produce OMVs.

[00164] The antigenic composition can contain microvesicles (MV) (or “blebs”) containing subject fHbps, where the MV or blebs are released during culture of a *Neisseria meningitidis* strain genetically modified to express a subject fHbp. For example, MVs may be obtained by culturing a strain of *Neisseria meningitidis* in broth culture medium, separating whole cells from the broth culture medium (e.g. by filtration, or by a low-speed centrifugation that pellets only the cells and not the smaller blebs, or the like), and then collecting the MVs that are present in the cell-free culture medium (e.g. by filtration, differential precipitation or aggregation of MVs, or by a high-speed centrifugation that pellets the blebs, or the like). Strains for use in production of MVs can generally be selected on the basis of the amount of blebs produced in culture (e.g., bacteria can be cultured in a reasonable number to provide for production of blebs suitable for isolation and administration in the methods described herein). An exemplary strain that produces high levels of blebs is described in PCT Publication No. WO 01/34642. In addition to bleb production, strains for use in MV production may also be selected on the basis of NspA production, where strains that produce higher levels of NspA may be of particular interest (for examples of *N. meningitidis* strains having different NspA production levels, see, e.g., Moe et al. (1999 *Infect. Immun.* 67: 5664). Other strains of

interest for use in production of blebs include strains having an inactivated GNA33 gene, which encodes a lipoprotein required for cell separation, membrane architecture and virulence (see, e.g., Adu-Bobie et al. (2004) *Infect Immun.*72:1914-1919).

[00165] The antigenic compositions of the present disclosure can contain vesicles from one strain, or from 2, 3, 4, 5 or more strains, which strains may be homologous or heterologous, usually heterologous, to one another. For example, the strains may be homologous or heterologous with respect to PorA and/or the fHbp from which the subject fHbp is derived. The vesicles can be prepared from strains that express more than one subject fHbp (e.g., 1, 2, 3, or more subject fHbp) which may be composed of fHbp amino acid sequences from different variants (v.1, v.2, or v.3) or subvariants (e.g., a subvariant of v.1, v.2, or v.3).

[00166] The antigenic compositions can comprise a mixture of OMVs and MVs presenting the same or different subject fHBps, where the subject fHBps may optionally present epitopes from different combinations of fHbp variants and/or subvariants and where the OMVs and/or MVs may be from the same or different strains. Vesicles from different strains can be administered as a mixture, or can be administered serially.

[00167] Where desired (e.g., where the strains used to produce vesicles are associated with endotoxin or particular high levels of endotoxin), the vesicles are optionally treated to reduce endotoxin, e.g., to reduce toxicity following administration. Although less desirable as discussed below, reduction of endotoxin can be accomplished by extraction with a suitable detergent (for example, BRIJ-96, sodium deoxycholate, sodium lauroylsarcosinate, Empigen BB, Triton X-100, TWEEN 20 (sorbitan monolaurate polyoxyethylene), TWEEN 80, at a concentration of 0.1-10%, preferably 0.5-2%, and SDS). Where detergent extraction is used, it is preferable to use a detergent other than deoxycholate.

[00168] The vesicles of the antigenic compositions can be prepared without detergent, e.g., without use of deoxycholate. Although detergent treatment is useful to remove endotoxin activity, it may deplete the native fHbp lipoprotein and/or subject fHbp (including lipidated fHbp) by extraction during vesicle production. Thus it may be particularly desirable to decrease endotoxin activity using technology that does not require a detergent. In one approach, strains that are relatively low producers of endotoxin (lipopolysaccharide, LPS) are used so as to avoid the need to remove endotoxin from the final preparation prior to use in humans. For example, the vesicles can be prepared from *Neisseria* mutants in which lipooligosaccharide or other antigens that may be undesirable in a vaccine (e.g. Rmp) is reduced or eliminated.

[00169] Vesicles can be prepared from *N. meningitidis* strains that contain genetic modifications that result in decreased or no detectable toxic activity of lipid A. For example, such strain can be genetically modified in lipid A biosynthesis (Steeghs et al. (1999) *Infect Immun* 67:4988-93; van der Ley et al. (2001) *Infect Immun* 69:5981-90; Steeghs et al. (2004) *J Endotoxin Res* 10:113-9; Fissaha et al, (2005) *Infect Immun* 73:4070). The immunogenic compositions may be detoxified by modification of LPS, such as downregulation and/or inactivation of the enzymes encoded by *lpxL1* or *lpxL2*, respectively. Production of a penta-acylated lipid A made in *lpxL1* mutants indicates that the enzyme encoded by *lpxL1* adds the C12 to the N-linked 3-OH C14 at the 2' position of GlcN II. The major lipid A species found in *lpxL2* mutants is tetra-acylated, indicating the enzyme encoded by *lpxL2* adds the other C12, i.e., to the N-linked 3-OH C14 at the 2 position of GlcN I. Mutations resulting in a decreased (or no) expression of these genes (or decreased or no activity of the products of these genes) result in altered toxic activity of lipid A (van der Ley et al. (2001) *Infect Immun* 69:5981-90). Tetra-acylated (*lpxL2* mutant) and penta acylated (*lpxL1* mutant) lipid A are less toxic than the wild-type lipid A. Mutations in the lipid A 4'-kinase encoding gene (*lpxK*) also decrease the toxic activity of lipid A. Of particular interest for use in production of vesicles (e.g., MV or OMV) are *N. meningitidis* strains genetically modified so as to provide for decreased or no detectable functional *LpxL1*-encoded protein, e.g., where the *Neisseria* bacterium (e.g., *N. meningitidis* strain) is genetically modified to provide for decreased or no activity of a gene product of the *lpxL1* gene. For example, the *Neisseria* bacterium can be genetically modified to have an *lpxL1* gene knockout, e.g., where the *lpxL1* gene is disrupted. See, e.g., US Patent Publication No. 2009/0035328. The *Neisseria* bacterium can be genetically modified to provide for decreased or no activity of a gene product of the *lpxL2* gene. The *Neisseria* bacterium can be genetically modified to provide for decreased or no activity of a gene product of the *lpxL1* gene and the *lpxL2* gene. Such vesicles provide for reduced toxicity as compared to *N. meningitidis* strains that are wild-type for LPS production, while retaining immunogenicity of subject fHbp.

[00170] LPS toxic activity can also be altered by introducing mutations in genes/loci involved in polymyxin B resistance (such resistance has been correlated with addition of aminoarabinose on the 4' phosphate of lipid A). These genes/loci could be *pmrE* that encodes a UDP-glucose dehydrogenase, or a region of antimicrobial peptide-resistance genes common to many enterobacteriaceae which could be involved in aminoarabinose synthesis and transfer. The gene *pmrF* that is present in this region encodes a dolicol-phosphate manosyl

transferase (Gunn J. S., Kheng, B. L., Krueger J., Kim K., Guo L., Hackett M., Miller S. I. 1998. *Mol. Microbiol.* 27: 1171-1182).

[00171] Mutations in the PhoP-PhoQ regulatory system, which is a phospho-relay two component regulatory system (e.g., PhoP constitutive phenotype, PhoPc), or low Mg⁺⁺ environmental or culture conditions (that activate the PhoP-PhoQ regulatory system) lead to the addition of aminoarabinose on the 4'-phosphate and 2-hydroxymyristate replacing myristate (hydroxylation of myristate). This modified lipid A displays reduced ability to stimulate E-selectin expression by human endothelial cells and TNF secretion from human monocytes.

[00172] Polymyxin B resistant strains are also suitable for use, as such strains have been shown to have reduced LPS toxicity (see, e.g., van der Ley et al. (1994) In: *Proceedings of the ninth international pathogenic Neisseria conference*. The Guildhall, Winchester, England). Alternatively, synthetic peptides that mimic the binding activity of polymyxin B may be added to the antigenic compositions to reduce LPS toxic activity (see, e.g., Rustici et al. (1993) *Science* 259:361-365; Porro et al. (1998) *Prog Clin Biol Res.*397:315-25).

[00173] Endotoxin can also be reduced through selection of culture conditions. For example, culturing the strain in a growth medium containing 0.1 mg-100 mg of aminoarabinose per liter medium provides for reduced lipid toxicity (see, e.g., WO 02/097646).

COMPOSITIONS AND FORMULATIONS

[00174] "Compositions", "antigen composition", "antigenic composition" or "immunogenic composition" is used herein as a matter of convenience to refer generically to compositions comprising a subject non-naturally occurring fHbp as disclosed herein, which subject fHbp may be optionally conjugated to further enhance immunogenicity. Compositions useful for eliciting antibodies, e.g., antibodies against *N. meningitidis*, e.g., bactericidal antibodies to *N. meningitidis*, in a human are specifically contemplated by the present disclosure.

[00175] Antigenic compositions contain an immunologically effective amount of a subject non-naturally occurring fHbp, and may further include other compatible components, as needed. Compositions of the present disclosure can contain one or more non-naturally occurring fHbps as described above.

[00176] Immunogenic compositions contemplated by the present disclosure include, but are not limited to, compositions comprising:

[00177] a) a non-naturally occurring fHbp as provided above; and

b) a pharmaceutically acceptable excipient,

[00178] where the fHbp can be provided as recombinant protein and/or in a vesicle-based composition (e.g., OMV).

[00179] The compositions can contain additional antigens of interest, such as Porin A, Porin B, NspA, pilin, or other Neisserial proteins. In addition, the antigen compositions of the invention can comprise additional Neisserial antigens such as those exemplified in PCT Publication Nos. WO 99/24578, WO 99/36544; WO 99/57280, WO 00/22430, and WO 00/66791, as well as antigenic fragments of such proteins.

[00180] The compositions may contain more than one type of fHbp, where each fHbps may present epitopes from different combinations of fHbp variants and/or subvariants. For example, the composition may contain the non-naturally occurring fHbp as described above and a naturally occurring v. 1 and/or v. 2 fHbp. In another example, the composition may contain the non-naturally occurring fHbp as described above and a mutant v. 1 and/or v. 2 fHbp, in particular where the mutant fHbp has decreased binding to human fH relative to the naturally occurring corresponding fHbp. Such mutants of v. 1 and v. 2 fHbp are described in US2011/0256180, which is herein incorporated by reference. The v.1 and/or v.2 fHbp amino acid sequences can be combined with the non-naturally occurring fHbp amino acid sequence of the present disclosure as separate entities in the compositions of the invention, or within a fusion polypeptide.

[00181] In certain cases, the composition may include a vesicle obtained from a *Neisseria* host cell that is genetically modified with a nucleic acid encoding the non-naturally occurring fHbp described above, such that the encoded non-naturally occurring fHbp is produced by the genetically modified host cell, wherein the vesicle comprises the encoded non-naturally occurring fHbp; and a pharmaceutically acceptable excipient.

[00182] The host cells may be further modified to express additional antigens of interest, such as Porin A, Porin B, NspA, pilin, or other Neisserial proteins. In addition, the antigen compositions of the invention can comprise additional Neisserial antigens such as those exemplified in PCT Publication Nos. WO 99/24578, WO 99/36544; WO 99/57280, WO 00/22430, and WO 00/66791, as well as antigenic fragments of such proteins.

[00183] By “immunologically effective amount” is meant that the administration of that amount to an individual, either in a single dose, as part of a series of the same or different antigenic compositions, is effective to elicit an antibody response effective for treatment or

prevention of a symptom of, or disease caused by, for example, infection by *Neisseria*, particularly *N. meningitidis*, more particularly Group B, C, or Y *N. meningitidis*. This amount varies depending upon the health and physical condition of the individual to be treated, age, the capacity of the individual's immune system to produce antibodies, the degree of protection desired, the formulation of the vaccine, the treating clinician's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

[00184] Dosage regimen may be a single dose schedule or a multiple dose schedule (*e.g.*, including booster doses) with a unit dosage form of the antigenic composition administered at different times. The term “unit dosage form,” as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the antigenic compositions of the present disclosure in an amount sufficient to produce the desired effect, which compositions are provided in association with a pharmaceutically acceptable excipient (*e.g.*, pharmaceutically acceptable diluent, carrier or vehicle). The antigenic composition may be administered in conjunction with other immunoregulatory agents.

[00185] Antigenic compositions can be provided in a pharmaceutically acceptable excipient, which can be a solution such as a sterile aqueous solution, often a saline solution, or they can be provided in powder form. Such excipients can be substantially inert, if desired.

[00186] In some embodiments, a subject immunogenic composition comprises a subject fHbp present in a vesicle. In some embodiments, a subject immunogenic composition comprises a subject fHbp present in an MV. In some embodiments, a subject immunogenic composition comprises a subject fHbp present in an OMV. In some embodiments, a subject immunogenic composition comprises a mixture of MV and OMV comprising a subject fHbp. Vesicles, such as MV and OMV, are described above.

[00187] The antigenic compositions can further contain an adjuvant. Examples of known suitable adjuvants that can be used in humans include, but are not necessarily limited to, alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v Tween 80™, 0.5% w/v Span 85), CpG-containing nucleic acid (where the cytosine is unmethylated), QS21, MPL, 3DMPL, extracts from *Aquilia*, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, and the like. For experimental animals, one can use Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-

MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip- almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic antigen or antigenic epitope thereof.

[00188] Further exemplary adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59TM (WO 90/14837; Chapter 10 in Vaccine design: the subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing MTP-PE) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBITM adjuvant system (RAS), (Ribi Immunochem, Hamilton, Mont.) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (DetoxTM); (2) saponin adjuvants, such as QS21 or StimulonTM (Cambridge Bioscience, Worcester, Mass.) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent e.g. WO 00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) e.g. GB-2230221, EP-A-0689454, optionally in the substantial absence of alum when used with pneumococcal saccharides e.g. WO 00/56358; (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions e.g. EP-A-0835318, EP-A-0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs (Krieg Vaccine 2000, 19, 618-622; Krieg Curr Opin Mol Ther 2001 3:15-24; Roman et al., Nat. Med, 1997, 3, 849-854; Weiner et al., PNAS USA, 1997, 94, 10833-10837; Davis et al, J. Immunol, 1998, 160, 810-876; Chu et al., J. Exp. Med, 1997, 186, 1623-1631; Lipford et al, Ear. J. Immunol., 1997, 27, 2340-2344; Moldoveanu e/

al., Vaccine, 1988, 16, 1216-1224; Krieg et al., Nature, 1995, 374, 546-549; Klinman et al., PNAS USA, 1996, 93, 2879-2883; Ballas et al, J. Immunol, 1996, 157, 1840-1845; Cowdery et al, J. Immunol, 1996, 156, 4570-4575; Halpern et al, Cell Immunol, 1996, 167, 72-78; Yamamoto et al, Jpn. J. Cancer Res., 1988, 79, 866-873; Stacey et al, J. Immunol., 1996, 157, 2116-2122; Messina et al, J. Immunol, 1991, 147, 1759-1764; Yi et al, J. Immunol, 1996, 157, 4918-4925; Yi et al, J. Immunol, 1996, 157, 5394-5402; Yi et al, J. Immunol, 1998, 160, 4755-4761; and Yi et al, J. Immunol, 1998, 160, 5898-5906; International patent applications WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581, i.e. containing at least one CG dinucleotide, where the cytosine is unmethylated; (8) a polyoxyethylene ether or a polyoxyethylene ester e.g. WO 99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO 01/21152); (10) a saponin and an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) (WO 00/62800); (11) an immunostimulant and a particle of metal salt e.g. WO 00/23105; (12) a saponin and an oil-in-water emulsion e.g. WO 99/11241; (13) a saponin (e.g. QS21)+3dMPL+IM2 (optionally+a sterol) e.g. WO 98/57659; (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutarninyl-L-alanine-2-(1'-2'-dipalmitoyl-- sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), etc. Adjuvants suitable for administration to a human are of particular interest.

[00189] The antigen compositions may contain other components, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium, carbonate, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

[00190] The concentration of the subject fHbp in a formulation can vary widely (e.g., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight) and will usually be selected primarily based on fluid volumes, viscosities, and

patient-based factors in accordance with the particular mode of administration selected and the patient's needs.

[00191] The subject fHbp-containing formulations can be provided in the form of a solution, suspension, tablet, pill, capsule, powder, gel, cream, lotion, ointment, aerosol or the like. It is recognized that oral administration can require protection of the compositions from digestion. This is typically accomplished either by association of the composition with an agent that renders it resistant to acidic and enzymatic hydrolysis or by packaging the composition in an appropriately resistant carrier. Means of protecting from digestion are well known in the art.

[00192] The fHbp-containing formulations can also be provided so as to enhance serum half-life of fHbp following administration. For example, where isolated fHbps are formulated for injection, the fHbp may be provided in a liposome formulation, prepared as a colloid, or other conventional techniques for extending serum half-life. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4,235,871, 4,501,728 and 4,837,028. The preparations may also be provided in controlled release or slow-release forms.

IMMUNIZATION

[00193] The present disclosure provides a method of inducing an immune response to at least one Neisserial strain in a mammalian host. The methods generally involve administering to an individual in need thereof an effective amount of a subject immunogenic composition. The present disclosure also provides non-naturally occurring factor H binding protein (fHbp) of the invention for use as medicaments (*e.g.* as immunogenic compositions or as vaccines) or as diagnostic reagents. The present disclosure further provides the use of nucleic acid or protein of the invention in the manufacture of a medicament for preventing Neisserial (*e.g.* meningococcal) infection in a mammal.

[00194] The fHbp-containing antigenic compositions are generally administered to a human subject that is at risk of acquiring a Neisserial disease so as to prevent or at least partially arrest the development of disease and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for therapeutic use will depend on, *e.g.*, the antigenic composition, the manner of administration, the weight and general state of health of the patient, and the judgment of the prescribing physician. Single or

multiple doses of the antigenic compositions may be administered depending on the dosage and frequency required and tolerated by the patient, and route of administration.

[00195] The fHbp-containing antigenic compositions are generally administered in an amount effective to elicit an immune response, particularly a humoral immune response, e.g., a bactericidal antibody response, in the host. As noted above, amounts for immunization will vary, and can generally range from about 1 μ g to 100 μ g per 70 kg patient, usually 5 μ g to 50 μ g/70 kg. Substantially higher dosages (e.g. 10 mg to 100 mg or more) may be suitable in oral, nasal, or topical administration routes. The initial administration can be followed by booster immunization of the same or different fHbp-containing antigenic composition. Usually vaccination involves at least one booster, more usually two boosters.

[00196] In general immunization can be accomplished by administration by any suitable route, including administration of the composition orally, nasally, nasopharyngeally, parenterally, enterically, gastrically, topically, transdermally, subcutaneously, intramuscularly, in tablet, solid, powdered, liquid, aerosol form, locally or systemically, with or without added excipients. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa. (1980).

[00197] An anti-fHbp immune response can be assessed by known methods (e.g. by obtaining serum from the individual before and after the initial immunization, and demonstrating a change in the individual's immune status, for example an immunoprecipitation assay, or an ELISA, or a bactericidal assay, or a Western blot, or flow cytometric assay, or the like).

[00198] The antigenic compositions can be administered to a human subject that is immunologically naive with respect to *Neisseria meningitidis*. In a particular embodiment, the subject is a human child about five years or younger, and preferably about two years old or younger, and the antigenic compositions are administered at any one or more of the following times: two weeks, one month, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 months, or one year or 15, 18, or 21 months after birth, or at 2, 3, 4, or 5 years of age.

[00199] It may be generally desirable to initiate immunization prior to the first sign of disease symptoms, or at the first sign of possible or actual exposure to infection or disease (e.g., due to exposure or infection by *Neisseria*).

METHODS OF SCREENING

[00200] In one example, a method of evaluating the efficacy of a subject fHbp in a vaccine composition involves: (a) immunizing a host animal (e.g., a non-human mammalian host animal, such as a rodent, e.g., a mouse) with a composition comprising a fHbp of the present disclosure; and (b) measuring levels of bactericidal antibodies in the host. The subject method may also include assessing the susceptibility of a host animal administered with a vaccine comprising a subject fHbp to a *Neisseria* bacterium.

[00201] In another example, the method can involve making and identifying antibodies elicited by the subject fHbp. The method involves isolating antibodies from the host animal that have binding affinity to the fHbp, contacting a bacterial cell with the isolated antibodies; and assessing binding of the antibody to the bacterial cell. Additional steps may include assessing the competitive binding of the antibody to fHbp with human factor H; assessing the bactericidal activity against a bacterial pathogen when the antibody is administered to an animal contracted with the bacterial pathogen. In some embodiments, the antibody is in an antibody population, and the method can further comprise: isolating one or more antibodies of the antibody population that bind the bacterial cell. A featured aspect is isolated antibody that is bactericidal against the bacterial cell, which may include, for example, complement-mediated bactericidal activity and/or opsonophagocytic activity capable of decreasing the viability of the bacteria in human blood.

[00202] Bacterial pathogens of particular interest are *N. meningitidis* of any or all variant groups, of diverse capsular groups, such as *N. meningitidis* Serogroup B, *N. meningitidis* Serogroup C, *N. meningitidis* Serogroup X, *N. meningitidis* Serogroup Y, *N. meningitidis* Serogroup W-135, and the like.

METHODS OF EVALUATING A RESPONSE TO A FHBP

[00203] The present disclosure provides methods for determining the likelihood that a fHbp will elicit a bactericidal response in an individual; and methods of evaluating a variant fHbp for suitability for inclusion in an immunogenic composition.

Determining the likelihood that a fHbp will elicit a bactericidal response

[00204] The present disclosure provides a method of determining the likelihood that a non-naturally occurring fHbp of the present disclosure (e.g., a fHbp present in a *Neisserial* vaccine) will elicit a bactericidal response in an individual to at least one *Neisseria meningitidis* strain. The method generally involves determining the ability of antibody,

present in serum obtained from an individual who has been immunized with a fHbp, to inhibit binding of fH to fHbp. Inhibition of binding of fH to fHbp by the antibody at a level that is at least about 10% higher, at least about 25%, at least about 50%, at least about 75%, at least about 2-fold, at least about 10-fold, at least about 50-fold, at least about 100-fold, or greater than 100-fold, than the level of inhibition of fH to fHbp by a control antibody that inhibits fH binding to fHbp but that does not generate a bactericidal response, indicates that the fHbp is likely to elicit a bactericidal response to at least one *Neisseria meningitidis* strain.

[00205] The degree of inhibition of binding of fH to fHbp by antibody elicited by immunization with a subject fHbp can be determined using an assay as described herein, or any other known assay. For example, the fH and/or the fHbp can comprise a detectable label, and inhibition of fH/fHbp binding can be assessed by detecting the amount of labelled component present in an fH/fHbp complex and/or detecting the amount of label present in free fH and/or free fHbp (e.g., fH or fHbp not in an fH/fHbp complex).

[00206] In one example, assays to assess fH binding to an fHbp involve use of fHbp immobilized on a support (e.g., fHbp immobilized on the well of a microtiter plate). A mixture of a fixed concentration of human fH with dilutions of the test antibodies (e.g., antiserum, e.g., from a human or non-human test animal (e.g., mouse) that has received an antibody-eliciting dosage of an immunogenic composition) are added to the wells and incubated for an amount of time sufficient to allow for antibody binding. After washing the wells, bound fH is detected with a specific anti-fH antiserum (e.g., goat or donkey) containing a labeled component, or a secondary labeled antibody (e.g., rabbit anti-goat or anti-donkey anti-serum). Percent inhibition of bound fH can be calculated by the amount of bound fH in the absence of added human or mouse antibody.

[00207] In another variation of such assays, binding of fH to live bacteria in the presence or absence of test antisera is assessed by flow cytometry. Bacterial cells are incubated with a fixed concentration of fH (e.g., detectably labeled fH) and different dilutions of test sera containing antibody. The bacteria are washed and bound fH is detected (e.g., as described above).

[00208] Thus, the ability of antiserum from an individual immunized with a subject fHbp to inhibit fH/fHbp binding serves as a surrogate for directly assessing bactericidal activity of the antiserum. A method of the present disclosure for determining the likelihood that a subject fHbp will elicit a bactericidal response in an individual can provide information to a clinician

or other medical personnel as to whether a particular immunogenic composition has been effective in eliciting a bactericidal response in an individual.

[00209] Immunized individuals can have a similar serum IgG anti-fHbp antibody titer by ELISA. Antisera that provides for overall better inhibition of fH binding is indicative of a more effective, better quality anti-fHbp antibody response and will confer greater protection. Thus, for example, if in comparing the anti-Neisserial antibody response in two individuals (by the anti-fHbp antibodies, i.e, a serum dilution of 1:10,000 inhibits compared to a dilution of 1:3000 by the other individual) the individual with the higher inhibitory activity has better quality anti-fHbp antibody that will confer greater protection. The fH inhibition assay is thus a surrogate for complement-mediated bactericidal titer assays, which complement-mediated bactericidal titer assays are generally more time consuming and difficult to measure than fH inhibition.

Evaluating a subject non-naturally occurring fHbp

[00210] The present disclosure provides methods of assessing or predicting the likelihood that a non-naturally occurring fHbp as disclosed herein will be efficacious in eliciting a bactericidal antibody response in an individual. The methods generally involve assessing the ability of antibody specific for the fHbp variant to inhibit binding of fH to fHbp. The strength of inhibition of binding of fH to fHbp by antibody elicited by immunizing with an fHbp variant positively correlates with bactericidal activity of antibody elicited to the fHbp variant. A fHbp variant that elicits antibody that inhibits binding of fH to fHbp at a high serum dilution is considered a suitable candidate for a vaccine for eliciting protection against one or more strains of *Neisseria*.

[00211] For example, the present disclosure provides a method of determining the likelihood that a non-naturally occurring fHbp that has lower affinity for human fH than fHbp ID 79 will elicit bactericidal antibodies in an individual to at least one *Neisseria meningitidis* strain. The method generally involves determining the ability of an antibody elicited in a test non-human animal to the non-naturally occurring fHbp to inhibit binding of fH to fHbp. Inhibition of binding of fH to fHbp by the antibody elicited to the non-naturally occurring fHbp at a level that is at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 2-fold, at least about 10-fold, at least about 50-fold, at least about 100-fold, or greater than 100-fold, higher than the level of inhibition of fH to fHbp by an antibody elicited in the test non-human animal to fHbp ID 79 indicates that the non-naturally occurring fHbp is likely to elicit a bactericidal response to at least one *Neisseria meningitidis* strain.

[00212] Suitable test non-human animals include, e.g., mice, rats, rabbits, and the like. The degree of inhibition of binding of fH to fHbp by antibody elicited to a fHbp variant can be determined using an assay as described herein, or any other known assay. Bactericidal activity of an antibody is readily determined using an assay as described herein, or any other known assay.

[00213] A subject method for determining the likelihood that a given non-naturally occurring fHbp that has lower affinity for human fH than fHbp ID 79 will elicit bactericidal antibodies in an individual to at least one *Neisseria meningitidis* strain is useful for identifying suitable immunogens (and/or eliminating unsuitable immunogens), e.g., in the course of vaccine development.

EXAMPLES

[00214] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLE 1: GENERATION OF MUTANT fHBP ID79

[00215] In order to generate mutants of v. 3 fHbp proteins having decreased binding to human factor H, H223A and H223R substitutions were introduced into fHbp ID 79. Figure 1 shows SDS-polyacrylamide gel indicating size and purity of recombinant factor H-binding protein ID 79 wild-type and mutants. The numbering of the histidine is based on the amino acid position with reference to the amino acid sequence of fHbp ID 1, as shown in the alignment provided in Figure 4. The histidine 223 position corresponds to histidine 230 in v. 3 fHbp (see Figures 4 and 5). The histidine 223 position corresponds to histidine 222 in v. 2 fHbp (e.g. fHbp ID 22; see Figure 4). Lane 1, Benchmark Ladder (Invitrogen); lane 2 ID 79 wild-type; lane 3 H223R mutant; lane 4, H223A mutant. 2 µg of each of the recombinant fHbps was loaded on the gel. A NuPAGE 4-12% polyacrylamide/Bis-Tris gel was used with MES Running Buffer (Invitrogen). The gel was stained with Simply Blue SafeStain (Invitrogen).

EXAMPLE 2: IDENTIFICATION OF V.3 fHBP MUTANT WITH DECREASED fH BINDING

[00216] Binding of human factor H to ID 79 mutants H223A and H223R by assayed by ELISA. Results are shown in Figure 2. Figure 2, Panel A shows binding of human factor H. fHbp ID 79 wild-type (WT), circular symbols; H223A mutant, triangular symbols; H223R mutant, asterisk symbols. Figure 2, Panel B shows binding of a control mAb, JAR 11.

[00217] Whereas the H223A mutant had only a slight decrease in binding, the H223R mutant showed no detectable binding to human factor H (Figure 2, Panel A). Binding of a control anti-fHbp mAb, JAR 11, to the H223A and H223R mutant proteins was similar to the wild-type fHbp ID 79 (Figure 2, Panel B).

[00218] Purified recombinant fHbp ID 79 wild-type or mutant proteins (2 µg/ml in PBS) were adsorbed to the wells of a microtiter plate. Non-specific binding was blocked with PBS/1% BSA. For data in Panel A, serial dilutions of purified human factor H (0.0012 to 20 µg/ml) were added to the wells and the plate was incubated for 1 h at room temperature. After washing, sheep anti-human factor H (1:2000 dilution; Panel A) or anti-fHbp mAb JAR 11 (0.00032-25 µg/ml; Panel B) were added to the wells and were incubated for 1 h at room temperature. After washing again, donkey anti-sheep IgG (1:5000; Sigma; Panel A) or goat anti-mouse IgG (1:5000; Sigma Panel B) was added and the plate was incubated for 1 h at room temperature. After washing again, phosphatase substrate (p-nitrophenyl phosphate, 1 mg/ml; Sigma) was added and the optical density at 405 nm was measured after 30 min incubation at room temperature.

EXAMPLE 3: fHBP MUTANT WITH DECREASED fH BINDING

[00219] T221A and H223A substitutions were introduced individually into fHbp ID 22 (variant group 2). The numbering of the T221 and H223 is based on the amino acid position with reference to the amino acid sequence of fHbp ID 1, as shown in the alignment provided in Figure 4. T221 corresponds to amino acid position 220 in v. 2 fHbp (e.g. fHbp ID 22) and H223 corresponds to amino acid position 222 in v. 2 fHbp (e.g. fHbp ID 22).

[00220] Whereas the T221A mutant showed no detectable binding to human factor H, the H223A mutant showed only a slight decrease in binding compared with the wild-type fHbp ID 22 protein (Figure 3, Panel A). Binding of a control anti-fHbp mAb, JAR 13, was similar for the wild-type fHbp ID 22 protein, T221A mutant, and H223A mutant (Figure 3, Panel B).

[00221] Binding of human factor H to ID 22 mutants T221A and H223A was assayed by ELISA. Figure 3, Panel A shows binding of human factor H. fHbp ID 22 wild-type (WT), circular symbols; T221A mutant, asterisk symbols; H223A mutant, triangular symbols. Figure 3, Panel B shows binding of a control mAb, JAR 13. Purified recombinant fHbp ID 22 wild-type or mutant proteins (2 µg/ml in PBS) were adsorbed to the wells of a microtiter plate. Non-specific binding was blocked with PBS/1% BSA. For data in Panel A, serial dilutions of purified human factor H (0.00032-25 µg/ml) were added to the wells and the plate was incubated for 1 h at room temperature. After washing, sheep anti-human factor H (1:2000 dilution; Panel A) or anti-fHbp mAb JAR 13 (0.00032-25 µg/ml; Panel B) were added to the wells and were incubated for 1 h at room temperature. After washing again, donkey anti-sheep IgG (1:5000; Sigma; Panel A) or goat anti-mouse IgG (1:5000; Sigma Panel B) was added and the plate was incubated for 1 h at room temperature. After washing again, phosphatase substrate (p-nitrophenyl phosphate, 1 mg/ml; Sigma) was added and the optical density at 405 nm was measured after 30 min incubation at room temperature.

EXAMPLE 4: BACERICIDAL RESPONSE

[00222] Wild-type CD-1 mice were immunized with 10 µg of recombinant fHbp ID 79 vaccines with aluminum hydroxide (600 µg per dose; Alhydrogel, Brenntag Biosector) as the adjuvant. A group of six animals was immunized with adjuvant alone (Alum) and groups of eight animals each were immunized with the recombinant fHbp vaccines. Three doses of vaccine were given at three-week intervals and blood was collected three weeks after the third dose. Serum bactericidal assays were performed using serum pools for the adjuvant control group (2 pools from 3 mice each) and individual sera for the fHbp vaccine groups. The bactericidal assay was performed using log-phase bacteria grown in Mueller-Hinton broth containing 0.25% glucose and 0.02 mM CMP-NANA, IgG-depleted human serum as a source of complement. The titers were defined as the reciprocal dilution of serum that resulted in 50% survival of the bacteria after incubation for 60 min relative to negative control wells containing serum from naïve mice. The results are shown in Figure 7.

CLAIMS**WHAT IS CLAIMED IS:**

1. A non-naturally occurring factor H binding protein (fHbp) derived from a naturally occurring variant 3 fHbp, the non-naturally fHbp comprising:

a substitution of the histidine at position 223 of the naturally occurring variant 3 fHbp with an amino acid selected from the group consisting of arginine, lysine, phenylalanine, tyrosine, or tryptophan, wherein the numbering of position 223 is based on the numbering of the mature fHbp ID 1,

wherein the non-naturally occurring fHbp has lower affinity for human factor H (fH) than fHbp ID 79.

2. The non-naturally occurring fHbp of claim 1, wherein the histidine is substituted with arginine.

3. The non-naturally occurring fHbp of any one of claims 1-2, wherein the variant 3 fHbp is a modular group V fHbp.

4. The non-naturally occurring fHbp of any one of claims 1-2, wherein the variant 3 fHbp is a modular group II fHbp.

5. The non-naturally occurring fHbp of any one of claims 1-3, wherein the non-naturally occurring fHbp comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of the fHbp ID 79.

6. The non-naturally occurring fHbp of any one of claims 1-5, wherein the non-naturally occurring fHbp comprises fHbp ID 28 comprising the amino acid substitution H223R.

7. The non-naturally occurring fHbp of any one of claims 1-5, wherein the non-naturally occurring fHbp comprises fHbp ID 67 or fHbp ID 175 comprising the amino acid substitution H223R.

8. The non-naturally occurring fHbp of any one of claims 1-5, wherein the non-naturally occurring fHbp comprises fHbp ID 79 comprising the amino acid substitution H223R.

9. The non-naturally occurring fHbp of any one of claims 1-5, wherein the non-naturally occurring fHbp comprises fHbp ID 45 comprising the amino acid substitution H223R.

10. An immunogenic composition comprising:

- a) the non-naturally occurring fHbp according to any of claims 1-9; and
- b) a pharmaceutically acceptable excipient.

11. The immunogenic composition of claim 10, wherein the non-naturally occurring fHbp is expressed on surface of a vesicle preparation prepared from a *Neisseria meningitidis* strain expressing the fHbp.

12. The immunogenic composition of claim 10, wherein the non-naturally occurring fHbp is present as an isolated polypeptide.

13. The immunogenic composition of any one of claims 11-12, wherein said pharmaceutically acceptable excipient comprises an adjuvant.

14. The immunogenic composition of any one of claims 10-13, further comprising an additional *N. meningitidis* antigen.

15. A method of eliciting an antibody response in a mammal, the method comprising administering to a mammal the non-naturally occurring fHbp according to any of claims 1-9, or the immunogenic composition of any of claims 10-14.

16. The method of claim 15, wherein said administering provides for production of bactericidal antibodies to *N. meningitidis*.

17. A nucleic acid encoding the fHbp according to any of claims 1-10.
18. A recombinant expression vector comprising the nucleic acid of claim 17.
19. A genetically modified host cell comprising the nucleic acid of claim 17 or the recombinant expression vector of claim 18.
20. An immunogenic composition comprising:
 - a) a vesicle obtained from a genetically modified *Neisseria* host cell that is genetically modified with a nucleic acid encoding the non-naturally occurring fHbp according to any of claims 1-9, such that the encoded non-naturally occurring fHbp is produced by the genetically modified host cell, wherein the vesicle comprises the encoded non-naturally occurring fHbp; and
 - b) a pharmaceutically acceptable excipient.
21. The immunogenic composition of claim 20, wherein the vesicle is a native outer membrane vesicle.
22. The immunogenic composition of any one of claims 20-21, wherein the host cell is genetically modified to provide for decreased or no activity of a polypeptide product of the *lpxL1* gene and/or the *lpxL2* gene.
23. The immunogenic composition of any one of claims 20-22, wherein the host cell is genetically modified to provide for increased expression of a *Neisserial* antigen.
24. A method of eliciting an antibody response in a mammal, the method comprising administering to a mammal the immunogenic composition of any one of claims 20-23.