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(54) **Titre : COMBINAISONS AVEC ADJUVANT DE PROTEINES MENINGOCOCCIQUES LIANT LE FACTEUR H**
(54) **Title: ADJUVANTED COMBINATIONS OF MENINGOCOCCAL FACTOR H BINDING PROTEINS**

(57) **Abrégé/Abstract:**
The M01573 sequence of meningococcal fHbp offers poor coverage in a vaccine. The invention addresses this poor coverage in two ways. In a first aspect, a fHbp-based vaccine includes two family I fHbp sequences, one which is more closely related to MC58 than to M01573, and vice versa. In a second aspect, a multi-family fHbp-based vaccine uses a family I fHbp sequence which is more closely related to MC58 than to M01573, in combination with a family III fHbp sequence. The compositions are adjuvanted with an aluminium phosphate adjuvant.

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(57) **Abstract**: The M01573 sequence of meningococcal fHbp offers poor coverage in a vaccine. The invention addresses this poor coverage in two ways. In a first aspect, a fHbp-based vaccine includes two family I fHbp sequences, one which is more closely related to MC58 than to M01573, and vice versa. In a second aspect, a multi-family fHbp-based vaccine uses a family I fHbp sequence which is more closely related to MC58 than to M01573, in combination with a family III fHbp sequence. The compositions are adjuvanted with an aluminium phosphate adjuvant.

ADJUVANTED COMBINATIONS OF MENINGOCOCCAL FACTOR H BINDING PROTEINS

TECHNICAL FIELD

The invention is in the field of meningococcal vaccines, in particular those containing fHbp.

BACKGROUND ART

Neisseria meningitidis (meningococcus) is a Gram-negative spherical bacterium. Current meningococcal vaccines are also based on capsular saccharides. These include monovalent serogroup C conjugate vaccines and 4-valent conjugate mixtures for serogroups A, C, W135 and Y. There is currently no useful vaccine authorised for general use against serogroup B ('MenB').

One antigen which has been proposed for use in immunising against MenB is the factor H binding protein ('fHbp'). This antigen has also been called protein '741' (SEQ IDs 2535 & 2536 in ref. 34), 'NMB1870', 'GNA1870' [refs. 1-3], 'P2086', 'LP2086' or 'ORF2086' [4-6]. The protein has been well studied. It is naturally a lipoprotein and is expressed across all meningococcal serogroups. The structure of fHbp's C-terminal immunodominant domain ('fHbpC') has been determined by NMR [7]. This part of the protein forms an eight-stranded β-barrel, whose strands are connected by loops of variable lengths. The barrel is preceded by a short α-helix and by a flexible N-terminal tail.

The fHbp antigen falls into three distinct variants or families [8] and it has been found that serum raised against a given family is bactericidal within the same family, but is not active against strains which express one of the other two families *i.e.* there is intra-family cross-protection, but not inter-family cross-protection. Thus reference 8 proposes to combine different variants of fHbp into a single vaccine composition, thereby increasing strain coverage, either as a mixture of separate proteins or as a fusion protein of the different variants (the latter being 'tandem proteins').

DISCLOSURE OF THE INVENTION

Family I of fHbp includes strain MC58, which is the strain which was used for the first genomic sequence publication [9] and is available from the ATCC as "BAA-335". The MC58 sequence for fHbp is SEQ ID NO: 1 herein, shown in SEQ ID NO: 26 starting at the cysteine at the N-terminus of the mature meningococcal protein. Family I also includes strain M01573 [5], whose sequence is SEQ ID NO: 23 herein (also referred to as the "B01" or "CDC1573" sequence; see database entry GI:40353481). SEQ ID NOs: 23 and 26 are aligned below.

Although the M01573 fHbp sequence is proposed for use in a human vaccine [10], and despite its close sequence relationship to fHbp from strain MC58, the inventors have found that serum raised against the M01573 sequence offers poor protection against the MC58 strain. As MC58 is representative of at least 30% of circulating family I strains, the M01573 sequence thus offers poor coverage of family I. It is an object of the invention to address this poor coverage, and to provide further and improved vaccine compositions which include fHbp antigens from multiple families. In broad terms, this object is achieved in two ways:

In a first aspect, a fHbp-based vaccine includes two family I fHbp sequences, one which is more closely related to MC58 (SEQ ID NO: 1) than to M01573 (SEQ ID NO: 23), and the other *vice versa*. Thus the invention provides an immunogenic composition which comprises a first fHbp antigen and a second fHbp antigen, wherein: the first fHbp antigen comprises an amino acid sequence which is more closely related to SEQ ID NO: 1 than to SEQ ID NO: 23; and the second fHbp antigen comprises an amino acid sequence which is more closely related to SEQ ID NO: 23 than to SEQ ID NO: 1. As explained in more detail below, this immunogenic composition can include further antigen components in addition to these two fHbp antigens.

In a second aspect, a multi-family fHbp-based vaccine uses a family I sequence which is more closely related to MC58 than to M01573, in combination with a family III sequence (*e.g.* SEQ ID NO: 25 from strain M01240320). Thus the invention provides an immunogenic composition which comprises a first fHbp antigen and a second fHbp antigen, wherein: the first fHbp antigen comprises an amino acid sequence which is more closely related to SEQ ID NO: 1 than to SEQ ID NO: 23; and the second fHbp antigen comprises an amino acid sequence (i) having at least *w*% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 25. The value of *w* is at least 94 (*e.g.* 95, 96, 97, 98, 99 or more). The value of *x* is either (a) at least 50 *e.g.* 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 180, 200 or (b) at least 7 (*e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200), provided that the fragment is not also a fragment of SEQ ID NO: 2 and/or of SEQ ID NO: 3. The first and second fHbp antigens have different amino acid sequences. As explained in more detail below, this immunogenic composition can include further antigen components in addition to these two fHbp antigens but, preferably, it does not include a fHbp antigen comprising an amino acid sequence with ≥95% identity to SEQ ID NO: 23.

Immunogenic compositions of the invention are adjuvanted. The adjuvant comprises an aluminium phosphate salt (see below).

Factor H binding protein(s)

Compositions of the invention include at least two different meningococcal factor H binding proteins (fHbp) to generate distinct immune responses which are not fully cross-reactive and which provide a broader spectrum of strain coverage against meningococci that the single fHbps alone.

In the invention's first aspect, a composition includes two family I fHbp antigen sequences. The first of these is more closely related to MC58 than to M01573; the second is more closely related to M01573 than to MC58. In the invention's aspect, a composition includes a fHbp which is more closely related to MC58 than to M01573. A fHbp antigen sequence may be defined as more closely related to MC58 than to M01573 if it meets one or more of the following criteria:

(a) it can elicit antibodies in a mouse which recognise a wild-type meningococcal fHbp comprising SEQ ID NO: 1 but which, under the same binding conditions, do not recognise a wild-type meningococcal fHbp comprising SEQ ID NO: 4 [NB: it may elicit some antibodies which recognise both SEQ ID NOs: 1 and 4].

(b) it can elicit antibodies in a mouse which are bactericidal against strain MC58 in a serum bactericidal assay; for example, providing a serum bactericidal titer of ≥1:4 using the Goldschneider assay with human complement [11-13], and/or providing a serum bactericidal titer of ≥1:128 using baby rabbit complement.

(c) it has at least 75% sequence identity to both of SEQ ID NO: 1 and SEQ ID NO: 23, but it has a higher sequence identity to SEQ ID NO: 1 than to SEQ ID NO: 23, when aligned using the same algorithm and parameters.

(d) it includes one or more of the 6-mers listed as SEQ ID NOs: 27 to 37. NB: the 6-mers of SEQ ID NOs: 27 to 34 may overlap in the sequence, and the 6-mers of SEQ ID NOs: 36 & 37 may overlap in the sequence.

(e) when aligned with SEQ ID NO: 1, it does not have a threonine residue (and preferably has an alanine residue) at the amino acid residue which aligns with Ala-2 of SEQ ID NO:1.

(f) when aligned with SEQ ID NO: 1, it does not have a valine residue (and preferably has a glycine residue) at the amino acid residue which aligns with Gly-141 of SEQ ID NO:1.

Of these six criteria, a sequence preferably meets at least criterion (b) or (c), more preferably at least (b) & (c), even more preferably at least (a), (b) & (c), and most preferably all six.

For the first aspect, a fHbp antigen sequence may be defined as more closely related to M01573 than to MC58 if it meets one or more of the following criteria:

(a) it can elicit antibodies in a mouse which recognise a wild-type meningococcal fHbp comprising SEQ ID NO: 4 but which, under the same binding conditions, do not recognise a wild-type meningococcal fHbp comprising SEQ ID NO: 1 [NB: it may elicit some antibodies which recognise both SEQ ID NOs: 1 and 4].

(b) it elicits antibodies in a mouse which are not bactericidal against strain MC58 in a serum bactericidal assay; for example, providing a serum bactericidal titer of ≤1:4 using the Goldschneider assay with human complement [11-13], and/or providing a serum bactericidal titer of ≤1:128 using baby rabbit complement.

(c) it has at least 75% sequence identity to both of SEQ ID NO: 1 and SEQ ID NO: 23, but it has a higher sequence identity to SEQ ID NO: 23 than to SEQ ID NO: 1, when aligned using the same algorithm and parameters.

(d) it includes one or more of the 6-mers listed as SEQ ID NOs: 38 to 48. NB: the 6-mers of SEQ ID NOs: 38 to 45 may overlap in the sequence, and the 6-mers of SEQ ID NOs: 47 & 48 may overlap in the sequence.

(e) when aligned with SEQ ID NO: 1, it has a threonine residue at the amino acid residue which aligns with Ala-2 of SEQ ID NO:1.

(f) when aligned with SEQ ID NO: 1, it has a valine residue at the amino acid residue which aligns with Gly-141 of SEQ ID NO:1.

Of these six criteria, a sequence preferably meets at least criterion (a) or (c), more preferably at least (a) & (c), even more preferably at least (a), (b) & (c), and most preferably all six.

For the second aspect of the invention, the second fHbp antigen comprises an amino acid sequence (i) having at least *w*% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 25. The value of *w* is at least 94 (*e.g.* 95, 96, 97, 98, 99 or more). The value of *x* is either (a) at least 50 *e.g.* 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 180, 200 or (b) at least 7 (*e.g.* 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200), provided that the fragment is not also a fragment of SEQ ID NO: 2 and/or of SEQ ID NO: 3. Preferred fragments comprise an epitope from SEQ ID NO: 25.

fHbp sequences which are more closely related to MC58 than to M01573, or *vice versa*, or which comprise a sequence with ≥94% identity to and/or a fragment of SEQ ID NO: 25, can be designed based on these known sequences, or they can be selected from the large number of wild-type fHbp sequences which have been reported in the art *e.g.* see references 1-8 and 14-19.

Suitable amino acid sequences used with the invention may, compared to SEQ ID NOs: 1 and 23, include one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) conservative amino acid replacements *i.e.* replacements of one amino acid with another which has a related side chain. Genetically-encoded amino acids are generally divided into four families: (1) acidic *i.e.* aspartate, glutamate; (2) basic *i.e.* lysine, arginine, histidine; (3) non-polar *i.e.* alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar *i.e.* glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In general, substitution of single amino acids within these families does not have a major effect on the biological activity. The antigens may have one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) single amino acid deletions relative to a reference sequence. The antigens may also include one or more (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, *etc.*) insertions (*e.g.* each of 1, 2, 3, 4 or 5 amino acids) relative to a reference sequence.

A composition of the invention can include only two different fHbp sequences, or can include more than two different fHbp sequences. For example, a composition of the first aspect may or may not

include further fHbp sequences beyond the two fHbp sequences discussed above *e.g.* it may include a fHbp sequence from family II and/or from family III *e.g.* SEQ ID NO: 2 and/or SEQ ID NO: 3 herein. Thus a composition may be a bivalent fHbp composition, or may include be more than two different fHbp sequences *e.g.* in a trivalent or tetravalent fHbp composition.

A composition of the first aspect comprises two family I fHbp sequences, and ideally also includes a fHbp sequence from family II and/or family III. Usefully, it includes a fHbp antigen comprising an amino acid sequence (i) having at least *w*% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 25. The values of *w* and *x* are given above, and preferred fragments comprise an epitope from SEQ ID NO: 25. Such fHbp antigens (also present in compositions of the second aspect) may elicit antibodies in a mouse which are bactericidal against strain 961-5945 in a serum bactericidal assay; for example, providing a serum bactericidal titer of ≥1:4 using the Goldschneider assay with human complement [11-13], and/or providing a serum bactericidal titer of ≥1:128 using baby rabbit complement. Strain 961-5945 has been widely reported in the literature (*e.g.* see refs. 1, 2 & 20-26) and is available as isolate MDU 9615945, id 1002, from the Neisseria PubMLST collection. It is a B:2b:P1.21,16 strain with electrophoretic type A4 and MLST type 153. Its fHbp sequence is given in reference 23, GI:106073476.

Thus a composition of the first aspect may comprise (i) first and second fHbp antigens as defined above, and (ii) a third fHbp antigen comprising an amino acid sequence (i) having at least *w*% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 25.

A composition of the second aspect comprises two different fHbp antigens as disclosed above. The composition may include a third fHbp antigen, but anyway it is preferred that the composition does not include (i) an antigen comprising an amino acid sequence which has >95% sequence identity to SEQ ID NO: 23 or (ii) an antigen comprising an amino acid sequence which has >95% sequence identity to SEQ ID NO: 24.

A fHbp antigen in a composition of the invention may be lipidated *e.g.* at a N-terminus cysteine. In other embodiments, however, fHbp antigen(s) are not lipidated. For lipidated fHbps, lipids attached to cysteines will usually include palmitoyl residues *e.g.* as tripalmitoyl-S-glyceryl-cysteine (Pam3Cys), dipalmitoyl-S-glyceryl cysteine (Pam2Cys), N-acetyl (dipalmitoyl-S-glyceryl cysteine), *etc.* Examples of mature lipidated fHbp sequences are SEQ ID NO: 23 (including SEQ ID NO: 4), SEQ ID NO: 24 (including SEQ ID NO: 5), and SEQ ID NO: 25 (including SEQ ID NO: 6).

Where a composition comprises different meningococcal fHbp antigens, these may be present as separate polypeptides (*e.g.* a first and second polypeptide) or they may be present as part of a single 'hybrid' polypeptide *i.e.* where at least two (*e.g.* 2, 3, 4, 5, or more) fHbp antigens are expressed as a single polypeptide chain (fusion protein), as disclosed for meningococcal antigens in reference 27.

Hybrid polypeptides can be represented by the formula NH_2-A-{-X-L-}$_n$-B-COOH, wherein: each X is an amino acid sequence of a different fHbp antigen as defined above; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; *n* is an integer of 2 or more (*e.g.* 2, 3, 4, 5, 6, *etc.*). Usually *n* is 2 or 3.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of {-X-L-}, linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where *n* = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID NO: 15) or GSGSGGGG (SEQ ID NO:16), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker. Another suitable linker, particularly for use as the final L_n is a Leu-Glu dipeptide.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine *e.g.* Met-Ala-Ser, or a single Met residue.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where *n* = 3, 4, 5, 6, 7, 8, 9, 10 or more, such as SEQ ID NO: 17), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Preferred compositions of the first and second aspects include a fHbp antigen comprising an amino acid sequence having at least 99% sequence identity (*e.g.* 100% identity) to SEQ ID NO: 25. This can be a third antigen in the first aspect and a second antigen in the second aspect. This antigen can be a lipoprotein with a N-terminal cysteine.

Adjuvants

As mentioned above, compositions of the invention are adjuvanted. The compositions include the adjuvant known as aluminium phosphate. This name is conventional, but is used for convenience only, as it is not a precise description of the actual chemical compound which is present (*e.g.* see chapter 9 of reference 28). The invention can use any of the "phosphate" adjuvants that are in general use.

The adjuvants known as "aluminium phosphate" are typically aluminium hydroxyphosphates, often also containing a small amount of sulfate (*i.e.* aluminium hydroxyphosphate sulfate). They may be obtained by precipitation, and the reaction conditions and concentrations during precipitation influence the degree of substitution of phosphate for hydroxyl in the salt. Aluminium phosphates generally have a PO_4/Al molar ratio between 0.3 and 1.2. Aluminium phosphates can be distinguished from strict $AlPO_4$ by the presence of hydroxyl groups. For example, an IR spectrum band at 3164cm^{-1} (*e.g.* when heated to 200°C) indicates the presence of structural hydroxyls. The aluminium phosphate adjuvant may contain a small amount of sulfate (*i.e.* aluminium hydroxyphosphate sulfate) and may also include sodium and/or chloride ions [29]. The adjuvant may be obtained by precipitation.

Aluminium hydroxyphosphate is not a stoichiometric compound and its hydroxyl and phosphate composition depends on precipitation reactants and conditions. This hydroxyl/phosphate composition affects the adjuvant's point of zero charge (PZC; the pH at which a surface has zero net charge). The PZC is inversely related to the degree of substitution of phosphate for hydroxyl (the P/Al molar ratio). Substitution of phosphate anions for hydroxyl anions lowers the PZC. Thus the PZC can be altered by changing the concentration of free phosphate ions in solution (more phosphate = more acidic PZC) or by adding a buffer such as a histidine buffer (makes PZC more basic). Aluminium phosphates used with the invention generally have a PZC of between 5.0 and 6.6 *e.g.* between 5.4 and 6.2.

The P/Al molar ratio of an aluminium phosphate adjuvant will generally be between 0.3 and 1.2, preferably between 0.8 and 1.2, or between 0.85 and 1.0, and more preferably about 0.9. A P/Al molar ratio of at least 0.5 can provide an adjuvant with better aging properties.

The aluminium phosphate will generally be amorphous (*i.e.* amorphous to X-rays). It will generally be particulate (*e.g.* plate-like morphology as seen in transmission electron micrographs). Typical diameters of the plates are 10-100nm, and these form aggregates sized 0.5-20μm (*e.g.* about

1-10μm). Adsorptive capacities of between 0.7-1.5 mg protein per mg Al^{+++} at pH 7.4 have been reported for aluminium phosphate adjuvants.

A typical adjuvant is amorphous aluminium hydroxyphosphate with P/Al molar ratio between 0.84 and 0.92, and this adjuvant may be included at 0.6mg Al^{3+}/ml.

The concentration of Al^{+++} in a composition for administration to a patient is preferably less than 5mg/ml *e.g.* ≤4 mg/ml, ≤3 mg/ml, ≤2 mg/ml, ≤1 mg/ml, *etc.* A preferred range is between 0.2 and 1mg/ml. A maximum Al^{+++} concentration of 0.85mg/dose is preferred.

It is known to use mixtures of different aluminium salts in a single vaccine *e.g.* see reference 30. Although adjuvants including both aluminium phosphate and hydroxide can be used with fHbp, it is preferred that a composition should not include any aluminium hydroxide adjuvant because, as described above, it can degrade certain antigens which may be admixed with the fHbp (in particular, conjugated bacterial capsular saccharides). Instead, it is preferred to use only an aluminium phosphate adjuvant.

When aluminium salt(s) is used as an adjuvant, at least 75% (by weight) of fHbp in a composition of the invention should be adsorbed to it/them *e.g.* ≥80%, ≥75% ≥90%, ≥95% or even 100%. The proportion of adsorbed fHbp can be controlled by altering salt concentration and/or pH during formulation *e.g.* in general, a higher NaCl concentration can decrease fHbp's adsorption. The amount of adsorption for any formulation will depend on a combination of parameters including the adjuvant's PZC, the salt concentration and pH during formulation, the adjuvant concentration, the antigen concentration and the antigen's pI. The impact of each of these parameters on adsorption can be readily assessed. The degree of adsorption can be determined by comparing the total amount of fHbp antigen in a composition (*e.g.* measured before adsorption occurs, or measured by desorbing adsorbed antigen) to the amount which remains in the supernatant after centrifugation (*e.g.* see chapter 4 of ref. 31). The absence of detectable antigen in the supernatant after centrifugation indicates that total adsorption has occurred *i.e.* all of the fHbp is in the pellet, which contains the insoluble adjuvant and its adsorbed content. Efficient adsorption of fHbp antigens can use the techniques disclosed in reference 32.

Further antigen(s)

In addition to fHbp antigen(s), compositions of the invention can include further antigens from meningococcus or from other pathogens *e.g.* from other bacteria such as pneumococcus.

Further meningococcal polypeptide antigens

In addition to including meningococcal fHbp antigens, a composition may include one or more further meningococcal polypeptide antigen(s). Thus a composition may include a polypeptide antigen selected from the group consisting of: 287, NadA, NspA, HmbR, NhhA, App, and/or Omp85. These antigens will usefully be present as purified polypeptides *e.g.* recombinant polypeptides. The antigen will preferably elicit bactericidal anti-meningococcal antibodies after administration to a subject. If a

composition includes a PorA antigen then, in some embodiments, only one meningococcal PorA serosubtype is included. In some embodiments, no meningococcal PorA outer membrane protein is included in a composition.

A composition of the invention may include a 287 antigen. The 287 antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB2132 (GenBank accession number GI:7227388; SEQ ID NO: 9 herein). The sequences of 287 antigen from many strains have been published since then. For example, allelic forms of 287 can be seen in Figures 5 and 15 of reference 33, and in example 13 and figure 21 of reference 34 (SEQ IDs 3179 to 3184 therein). Various immunogenic fragments of the 287 antigen have also been reported. Preferred 287 antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 9, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. The most useful 287 antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 9. Advantageous 287 antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include a NadA antigen. The NadA antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB1994 (GenBank accession number GI:7227256; SEQ ID NO: 10 herein). The sequences of NadA antigen from many strains have been published since then, and the protein's activity as a Neisserial adhesin has been well documented. Various immunogenic fragments of NadA have also been reported. Preferred NadA antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 10; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 10, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 10. The most useful NadA antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 10. Advantageous NadA antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject. A useful NadA antigen for use with the invention has amino acid sequence SEQ ID NO: 49.

A composition of the invention may include a NspA antigen. The NspA antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB0663 (GenBank accession number GI:7225888; SEQ ID NO: 11 herein). The antigen was previously known from references 35 & 36. The sequences of NspA antigen from many strains have been

published since then. Various immunogenic fragments of NspA have also been reported. Preferred NspA antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 11, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 11. The most useful NspA antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 11. Advantageous NspA antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

Compositions of the invention may include a meningococcal HmbR antigen. The full-length HmbR sequence was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB1668 (SEQ ID NO: 7 herein). Reference 37 reports a HmbR sequence from a different strain (SEQ ID NO: 8 herein). SEQ ID NOs: 7 and 8 differ in length by 1 amino acid and have 94.2% identity. The invention can use a polypeptide that comprises a full-length HmbR sequence, but it will often use a polypeptide that comprises a partial HmbR sequence. Thus in some embodiments a HmbR sequence used according to the invention may comprise an amino acid sequence having at least *i%* sequence identity to SEQ ID NO: 7, where the value of *i* is 50, 60, 70, 80, 90, 95, 99 or more. In other embodiments a HmbR sequence used according to the invention may comprise a fragment of at least *j* consecutive amino acids from SEQ ID NO: 7, where the value of *j* is 7, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more. In other embodiments a HmbR sequence used according to the invention may comprise an amino acid sequence (i) having at least *i%* sequence identity to SEQ ID NO: 7 and/or (ii) comprising a fragment of at least *j* consecutive amino acids from SEQ ID NO: 7. Preferred fragments of *j* amino acids comprise an epitope from SEQ ID NO: 7. Such epitopes will usually comprise amino acids that are located on the surface of HmbR. Useful epitopes include those with amino acids involved in HmbR's binding to haemoglobin, as antibodies that bind to these epitopes can block the ability of a bacterium to bind to host haemoglobin. The topology of HmbR, and its critical functional residues, were investigated in reference 38. The most useful HmbR antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 7. Advantageous HmbR antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include a NhhA antigen. The NhhA antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB0992 (GenBank accession number GI:7226232; SEQ ID NO: 12 herein). The sequences of NhhA antigen from many strains have been published since *e.g.* refs 33 & 39, and various immunogenic fragments of NhhA have been reported. It is also known as Hsf. Preferred NhhA antigens for use with the

invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 12; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 12, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 12. The most useful NhhA antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 12. Advantageous NhhA antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include an App antigen. The App antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB1985 (GenBank accession number GI:7227246; SEQ ID NO: 13 herein). The sequences of App antigen from many strains have been published since then. Various immunogenic fragments of App have also been reported. Preferred App antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 13, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. The most useful App antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 13. Advantageous App antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

A composition of the invention may include an Omp85 antigen. The Omp85 antigen was included in the published genome sequence for meningococcal serogroup B strain MC58 [9] as gene NMB0182 (GenBank accession number GI:7225401; SEQ ID NO: 14 herein). The sequences of Omp85 antigen from many strains have been published since then. Further information on Omp85 can be found in references 40 and 41. Various immunogenic fragments of Omp85 have also been reported. Preferred Omp85 antigens for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 14; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 14, wherein 'n' is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 14. The most useful Omp85 antigens of the invention can elicit antibodies which, after administration to a subject, can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 14. Advantageous Omp85 antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

Meningococcal lipooligosaccharide

In addition to including meningococcal fHbp polypeptide antigen(s), a composition may include one or more meningococcal lipooligosaccharide (LOS) antigen(s). Meningococcal LOS is a glucosamine-based phospholipid that is found in the outer monolayer of the outer membrane of the bacterium. It includes a lipid A portion and a core oligosaccharide region, with the lipid A portion acting as a hydrophobic anchor in the membrane. Heterogeneity within the oligosaccharide core generates structural and antigenic diversity among different meningococcal strains, which has been used to subdivide the strains into 12 immunotypes (L1 to L12). The invention may use LOS from any immunotype *e.g.* from L1, L2, L3, L4, L5, L6, L7 and/or L8.

The L2 and L3 α-chains naturally include lacto-N-neotetraose (LNnT). Where the invention uses LOS from a L2 or L3 immunotype this LNnT may be absent. This absence can be achieved conveniently by using mutant strains that are engineered to disrupt their ability to synthesise the LNnT tetrasaccharide within the α-chain. It is known to achieve this goal by knockout of the enzymes that are responsible for the relevant biosynthetic additions [42,43]. For instance, knockout of the LgtB enzyme prevents addition of the terminal galactose of LNnT, as well as preventing downstream addition of the α-chain's terminal sialic acid. Knockout of the LgtA enzyme prevents addition of the N-acetyl-glucosamine of LNnT, and also the downstream additions. LgtA knockout may be accompanied by LgtC knockout. Similarly, knockout of the LgtE and/or GalE enzyme prevents addition of internal galactose, and knockout of LgtF prevents addition of glucose to the Hep^{I} residue. Any of these knockouts can be used, singly or in combination, to disrupt the LNnT tetrasaccharide in a L2, L3, L4, L7 or L9 immunotype strain. Knockout of at least LgtB is preferred, as this provides a LOS that retains useful immunogenicity while removing the LNnT epitope.

In addition to, or in place of, mutations to disrupt the LNnT epitope, a knockout of the *galE* gene also provides a useful modified LOS, and a lipid A fatty transferase gene may similarly be knocked out [44]. At least one primary O-linked fatty acid may be removed from LOS [45].LOS having a reduced number of secondary acyl chains per LOS molecule can also be used [46].The LOS will typically include at least the GlcNAc-Hep_2phosphoethanolamine-KDO_2-Lipid A structure [47]. The LOS may include a GlcNAcβ1-3Galβ1-4Glc trisaccharide while lacking the LNnT tetrasaccharide.

LOS may be included in compositions of the invention in various forms. It may be used in purified form on its own. It may be conjugated to a carrier protein. When LOS is conjugated, conjugation may be via a lipid A portion in the LOS or by any other suitable moiety *e.g.* its KDO residues. If the lipid A moiety of LOS is absent then such alternative linking is required. Conjugation techniques for LOS are known from *e.g.* references 45, 47, 48, 49, *etc.* Useful carrier proteins for these conjugates are discussed below *e.g.* bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof.

The LOS may be from a strain (*e.g.* a genetically-engineered meningococcal strain) which has a fixed (*i.e.* not phase variable) LOS immunotype as described in reference 50. For example, L2 and

L3 LOS immunotypes may be fixed. Such strains may have a rate of switching between immunotypes that is reduced by more than 2-fold (even >50_fold) relative to the original wild-type strain. Reference 50 discloses how this result can be achieved by modification of the *lgtA* and/or *lgtG* gene products.

LOS may be O-acetylated on a GlcNac residue attached to its Heptose II residue *e.g.* for L3 [51].

An immunogenic composition can include more than one type of LOS *e.g.* LOS from meningococcal immunotypes L2 and L3. For example, the LOS combinations disclosed in reference 52 may be used.

A LOS antigen can preferably elicit bactericidal anti-meningococcal antibodies after administration to a subject.

However, preferred compositions of the invention are free from meningococcal lipooligosaccharide.

Meningococcal capsular saccharide antigen(s)

In addition to including meningococcal fHbp antigens, a composition may include one or more meningococcal capsular saccharide conjugates. A composition of the invention may include one or more conjugates of capsular saccharides from 1, 2, 3, or 4 of meningococcal serogroups A, C, W135 and Y *e.g.* A+C, A+W135, A+Y, C+W135, C+Y, W135+Y, A+C+W135, A+C+Y, A+W135+Y, A+C+W135+Y, *etc.* Compositions including a conjugated serogroup C capsular saccharide are useful, and compositions including saccharides from all of serogroups A, C, W135 and Y are ideal.

The capsular saccharide of serogroup A meningococcus is a homopolymer of (α1→6)-linked *N*-acetyl-D-mannosamine-1-phosphate, with partial O-acetylation in the C3 and C4 positions. Acetylation at the C-3 position can be 70-95%. Conditions used to purify the saccharide can result in de-O-acetylation (*e.g.* under basic conditions), but it is useful to retain OAc at this C-3 position. In some embodiments, at least 50% (*e.g.* at least 60%, 70%, 80%, 90%, 95% or more) of the mannosamine residues in a serogroup A saccharides are O-acetylated at the C-3 position. Acetyl groups can be replaced with blocking groups to prevent hydrolysis [53], and such modified saccharides are still serogroup A saccharides within the meaning of the invention.

The serogroup C capsular saccharide is a homopolymer of (α 2→9)-linked sialic acid (*N*-acetyl neuraminic acid, or 'NeuNAc'). The saccharide structure is written as →9)-Neu *p* NAc 7/8 OAc-(α2→ . Most serogroup C strains have O-acetyl groups at C-7 and/or C-8 of the sialic acid residues, but about 15% of clinical isolates lack these O-acetyl groups [54,55].The presence or absence of OAc groups generates unique epitopes, and the specificity of antibody binding to the saccharide may affect its bactericidal activity against O-acetylated (OAc+) and de-O-acetylated (OAc–) strains [56-58]. Serogroup C saccharides used with the invention may be prepared from either OAc+ or OAc– strains. Licensed MenC conjugate vaccines include both OAc– (NEISVAC-C™) and OAc+ (MENJUGATE™ & MENINGITEC™) saccharides. In some embodiments, strains for production of serogroup C conjugates are OAc+ strains, *e.g.* of serotype 16, serosubtype P1.7a,1, *etc.*. Thus

C:16:P1.7a,1 OAc+ strains may be used. OAc+ strains in serosubtype P1.1 are also useful, such as the C11 strain.

The serogroup W135 saccharide is a polymer of sialic acid-galactose disaccharide units. Like the serogroup C saccharide, it has variable O-acetylation, but at sialic acid 7 and 9 positions [59]. The structure is written as: →4)-D-Neu*p*5Ac(7/9OAc)-α-(2→6)-D-Gal-α-(1→ .

The serogroup Y saccharide is similar to the serogroup W135 saccharide, except that the disaccharide repeating unit includes glucose instead of galactose. Like serogroup W135, it has variable O-acetylation at sialic acid 7 and 9 positions [59]. The serogroup Y structure is written as: →4)-D-Neu*p*5Ac(7/9OAc)-α-(2→6)-D-Glc-α-(1→ .

The saccharides used according to the invention may be O-acetylated as described above (*e.g.* with the same O-acetylation pattern as seen in native capsular saccharides), or they may be partially or totally de-O-acetylated at one or more positions of the saccharide rings, or they may be hyper-O-acetylated relative to the native capsular saccharides.

The saccharide moieties in conjugates may comprise full-length saccharides as prepared from meningococci, and/or may comprise fragments of full-length saccharides *i.e.* the saccharides may be shorter than the native capsular saccharides seen in bacteria. The saccharides may thus be depolymerised, with depolymerisation occurring during or after saccharide purification but before conjugation. Depolymerisation reduces the chain length of the saccharides. One depolymerisation method involves the use of hydrogen peroxide. Hydrogen peroxide is added to a saccharide (*e.g.* to give a final H_2O_2 concentration of 1%), and the mixture is then incubated (*e.g.* at about 55°C) until a desired chain length reduction has been achieved. Another depolymerisation method involves acid hydrolysis. Other depolymerisation methods are known in the art. The saccharides used to prepare conjugates for use according to the invention may be obtainable by any of these depolymerisation methods. Depolymerisation can be used in order to provide an optimum chain length for immunogenicity and/or to reduce chain length for physical manageability of the saccharides. In some embodiments, saccharides have the following range of average degrees of polymerisation (Dp): A=10-20; C=12-22; W135=15-25; Y=15-25. In terms of molecular weight, rather than Dp, useful ranges are, for all serogroups: <100kDa; 5kDa-75kDa; 7kDa-50kDa; 8kDa-35kDa; 12kDa-25kDa; 15kDa-22kDa.

In some embodiments, the average molecular weight for saccharides from each of meningococcal serogroups A, C, W135 and Y may be more than 50kDa *e.g.* ≥75kDa, ≥100kDa, ≥110kDa, ≥120kDa, ≥130kDa, *etc.* [60], and even up to 1500kDa, in particular as determined by MALLS. For instance: a MenA saccharide may be in the range 50-500kDa *e.g.*60-80kDa; a MenC saccharide may be in the range 100-210kDa; a MenW135 saccharide may be in the range 60-190kDa *e.g.*120-140kDa; and/or a MenY saccharide may be in the range 60-190kDa *e.g.*150-160kDa.

The mass of meningococcal saccharide per serogroup in a composition will usually be between 1μg and 20μg *e.g.* between 2 and 10 μg per serogroup, or about 4μg or about 5μg or about 10μg. Where conjugates from more than one serogroup are included then they may be present at substantially equal masses *e.g.* the mass of each serogroup's saccharide is within +10% of each other. As an alternative to an equal ratio, a double mass of serogroup A saccharide may be used. Thus a vaccine may include MenA saccharide at 10μg and MenC, W135 and Y saccharides at 5μg each.

Useful carrier proteins for meningococcal conjugates include bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof. These are commonly used in conjugate vaccines. For example, the CRM197 diphtheria toxin mutant is useful [61]. Other suitable carrier proteins include synthetic peptides [62,63], heat shock proteins [64,65], pertussis proteins [66,67], cytokines [68], lymphokines [68], hormones [68], growth factors [68], artificial proteins comprising multiple human $CD4^+$ T cell epitopes from various pathogen-derived antigens [69] such as N19 [70], protein D from *H.influenzae* [71-73], pneumolysin [74] or its non-toxic derivatives [75], pneumococcal surface protein PspA [76], iron-uptake proteins [77], toxin A or B from *C.difficile* [78], recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) [79], *etc.* CRM197 is preferred.

Where a composition includes conjugates from more than one meningococcal serogroup it is possible to use the same carrier protein for each separate conjugate, or to use different carrier proteins. In both cases, though, a mixture of different conjugates will usually be formed by preparing each serotype conjugate separately, and then mixing them to form a mixture of separate conjugates.

Conjugates with a saccharide:protein ratio (w/w) of between 1:5 (*i.e.* excess protein) and 5:1 (*i.e.* excess saccharide) may be used *e.g.* ratios between 1:2 and 5:1 and ratios between 1:1.25 and 1:2.5. As described in reference 80, different meningococcal serogroup conjugates in a mixture can have different saccharide:protein ratios *e.g.* one may have a ratio of between 1:2 & 1:5, whereas another has a ratio between 5:1 & 1:1.99.

A carrier protein may be covalently conjugated to a meningococcal saccharide directly or via a linker. Various linkers are known. For example, attachment may be via a carbonyl, which may be formed by reaction of a free hydroxyl group of a modified saccharide with CDI [81,82] followed by reaction with a protein to form a carbamate linkage. Carbodiimide condensation can be used [83]. An adipic acid linker can be used, which may be formed by coupling a free $-NH_2$ group (*e.g.* introduced to a saccharide by amination) with adipic acid (using, for example, diimide activation), and then coupling a protein to the resulting saccharide-adipic acid intermediate [84,85]. Other linkers include β-propionamido [86], nitrophenyl-ethylamine [87], haloacyl halides [88], glycosidic linkages [89], 6-aminocaproic acid [90], N-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) [91], adipic acid dihydrazide ADH [92], C_4 to C_{12} moieties [93], *etc.*

Conjugation via reductive amination can be used. The saccharide may first be oxidised with periodate to introduce an aldehyde group, which can then form a direct covalent linkage to a carrier

protein via reductive amination *e.g.* to the ε-amino group of a lysine. If the saccharide includes multiple aldehyde groups per molecule then this linkage technique can lead to a cross-linked product, where multiple aldehydes react with multiple carrier amines.

As described in reference 94, a mixture can include one conjugate with direct saccharide/protein linkage and another conjugate with linkage via a linker. This arrangement applies particularly when using saccharide conjugates from different meningococcal serogroups *e.g.* MenA and MenC saccharides may be conjugated via a linker, whereas MenW135 and MenY saccharides may be conjugated directly to a carrier protein.

A meningococcal saccharide may comprise a full-length intact saccharide as prepared from meningococcus, and/or may comprise fragments of full-length saccharides *i.e.* the saccharides may be shorter than the native capsular saccharides seen in bacteria. The saccharides may thus be depolymerised, with depolymerisation occurring during or after saccharide purification but before conjugation. Depolymerisation reduces the chain length of the saccharides. Depolymerisation can be used in order to provide an optimum chain length for immunogenicity and/or to reduce chain length for physical manageability of the saccharides.

Conjugated pneumococcal capsular saccharide(s)

Compositions of the invention may include a pneumococcal capsular saccharide conjugated to a carrier protein.

The invention can include capsular saccharide from one or more different pneumococcal serotypes. Where a composition includes saccharide antigens from more than one serotype, these are preferably prepared separately, conjugated separately, and then combined. Methods for purifying pneumococcal capsular saccharides are known in the art (*e.g.* see reference 95) and vaccines based on purified saccharides from 23 different serotypes have been known for many years. Improvements to these methods have also been described *e.g.* for serotype 3 as described in reference 96, or for serotypes 1, 4, 5, 6A, 6B, 7F and 19A as described in reference 97.

Pneumococcal capsular saccharide(s) will typically be selected from the following serotypes: 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and/or 33F. Thus, in total, a composition may include a capsular saccharide from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or more different serotypes.

A useful combination of serotypes is a 7-valent combination *e.g.* including capsular saccharide from each of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Another useful combination is a 9-valent combination *e.g.* including capsular saccharide from each of serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F and 23F. Another useful combination is a 10-valent combination *e.g.* including capsular saccharide from each of serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. An 11-valent combination may further include saccharide from serotype 3. A 12-valent combination may add to the 10-valent mixture: serotypes 6A and 19A; 6A and 22F; 19A and 22F; 6A and 15B; 19A and 15B; or 22F and

15B. A 13-valent combination may add to the 11-valent mixture: serotypes 19A and 22F; 8 and 12F; 8 and 15B; 8 and 19A; 8 and 22F; 12F and 15B; 12F and 19A; 12F and 22F; 15B and 19A; 15B and 22F; 6A and 19A, *etc.*

Thus a useful 13-valent combination includes capsular saccharide from serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19, 19F and 23F *e.g.* prepared as disclosed in references 98 to 101. One such combination includes serotype 6B saccharide at about 8μg/ml and the other 12 saccharides at concentrations of about 4μg/ml each. Another such combination includes serotype 6A and 6B saccharides at about 8μg/ml each and the other 11 saccharides at about 4μg/ml each.

Suitable carrier proteins for conjugates are discussed above in relation to meningococcal conjugates. Particularly useful carrier proteins for pneumococcal conjugate vaccines are CRM197, tetanus toxoid, diphtheria toxoid and *H.influenzae* protein D. CRM197 is used in PREVNAR™. A 13-valent mixture may use CRM197 as the carrier protein for each of the 13 conjugates, and CRM197 may be present at about 55-60μg/ml.

Where a composition includes conjugates from more than one pneumococcal serotype, it is possible to use the same carrier protein for each separate conjugate, or to use different carrier proteins. In both cases, though, a mixture of different conjugates will usually be formed by preparing each serotype conjugate separately, and then mixing them to form a mixture of separate conjugates. Reference 102 describes potential advantages when using different carrier proteins in multivalent pneumococcal conjugate vaccines, but the PREVNAR™ product successfully uses the same carrier for each of seven different serotypes.

A carrier protein may be covalently conjugated to a pneumococcal saccharide directly or via a linker, as discussed above in relation to meningococcal conjugates. Cross-linking conjugation techniques are particularly useful for at least pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.

As discussed above for meningococcal saccharides, a pneumococcal saccharide may comprise a full-length intact saccharide as prepared from pneumococcus, and/or may comprise fragments of full-length saccharides. Where more than one pneumococcal serotype is used then it is possible to use intact saccharides for each serotype, fragments for each serotype, or to use intact saccharides for some serotypes and fragments for other serotypes. Where a composition includes saccharide from any of serotypes 4, 6B, 9V, 14, 19F and 23F, these saccharides are preferably intact. In contrast, where a composition includes serotype 18C saccharide it is preferably depolymerised.

A serotype 3 saccharide may also be depolymerised, For instance, a serotype 3 saccharide can be subjected to acid hydrolysis for depolymerisation [98] *e.g.* using acetic acid. The resulting fragments may then be oxidised for activation (*e.g.* periodate oxidation, maybe in the presence of bivalent cations *e.g.* with $MgCl_2$), conjugated to a carrier (*e.g.* CRM197) under reducing conditions (*e.g.* using sodium cyanoborohydride), and then (optionally) any unreacted aldehydes in the saccharide

can be capped (*e.g.* using sodium borohydride) [98]. Conjugation may be performed on lyophilized material *e.g.* after co-lyophilizing activated saccharide and carrier.

A serotype 1 saccharide may be at least partially de-O-acetylated *e.g.* achieved by alkaline pH buffer treatment [99] such as by using a bicarbonate/carbonate buffer. Such (partially) de-O-acetylated saccharides can be oxidised for activation (*e.g.* periodate oxidation), conjugated to a carrier (*e.g.* CRM197) under reducing conditions (*e.g.* using sodium cyanoborohydride), and then (optionally) any unreacted aldehydes in the saccharide can be capped (*e.g.* using sodium borohydride) [99]. Conjugation may be performed on lyophilized material *e.g.* after co-lyophilizing activated saccharide and carrier.

A serotype 19A saccharide may be oxidised for activation (*e.g.* periodate oxidation), conjugated to a carrier (*e.g.* CRM197) in DMSO under reducing conditions, and then (optionally) any unreacted aldehydes in the saccharide can be capped (*e.g.* using sodium borohydride) [103]. Conjugation may be performed on lyophilized material *e.g.* after co-lyophilizing activated saccharide and carrier.

Pneumococcal conjugates can ideally elicit anticapsular antibodies that bind to the relevant saccharide *e.g.* elicit an anti-saccharide antibody level $\geq$0.20μg/mL [104]. The antibodies may be evaluated by enzyme immunoassay (EIA) and/or measurement of opsonophagocytic activity (OPA). The EIA method has been extensively validated and there is a link between antibody concentration and vaccine efficacy.

Further antigens from other pathogen(s)

Compositions of the invention can include antigen(s) from further pathogen(s). For example, the composition may comprise one or more of the following further antigen(s):

- an antigen from hepatitis B virus, such as the surface antigen HBsAg.
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3.
- a diphtheria antigen, such as a diphtheria toxoid.
- a tetanus antigen, such as a tetanus toxoid.
- a saccharide antigen from *Haemophilus influenzae* B (Hib), typically conjugated.
- inactivated poliovirus antigen(s).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. DTP combinations are thus preferred.

Extemporaneous preparation

The invention also provides a kit comprising: (i) a first component comprising fHbp antigens, as described above; and (ii) a second component comprising a non-meningococcal immunogen. The kit components can be mixed to give an immunogenic composition for administering to a patient to protect against multiple pathogens.

The invention also provides a method for preparing a combined vaccine, comprising a step of mixing: (i) a first component comprising fHbp antigens, as described above; and (ii) a second component comprising a non-meningococcal immunogen. The mixed material may then be administered to a patient. The second component may be lyophilised, such that an aqueous first component reconstitutes it.

Pharmaceutical compositions

The invention is concerned with immunogenic compositions for administration to a patient. These compositions are pharmaceutically acceptable and will typically include a suitable carrier. A thorough discussion of pharmaceutically acceptable carriers is available in reference 105.

Effective dosage volumes can be routinely established, but a typical human unit dose of the composition has a volume of about 0.5ml.

The total amount of a fHbp polypeptide in a unit dose will usually be between 1 and 500μg/dose *e.g.* between 60 and 200μg/dose or between 120 and 500μg/ml. An amount of 20, 40, 50, 60, 80, 100 or 200μg for each fHbp antigen is typical in a human vaccine dose. Thus a vaccine may be formulated to include this amount of each fHbp.

The pH of a composition of the invention is usually between 6 and 8, and more preferably between 6.5 and 7.5 (*e.g.* about 7). As already discussed above, compositions may include a buffer *e.g.* a Tris buffer, a citrate buffer, a succinate buffer (such as a sodium succinate buffer), or a histidine buffer.

The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions of the invention may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2 mg/ml NaCl is typical *e.g.* about 9 mg/ml.

Compositions of the invention for administration to patients are immunogenic, and are more preferably vaccine compositions. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated

(*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor'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. The antigen content of compositions of the invention will generally be expressed in terms of the amount of protein per dose.

Meningococci affect various areas of the body and so the compositions of the invention may be prepared in various liquid forms. For example, the compositions may be prepared as injectables, either as solutions or suspensions. The composition may be prepared for pulmonary administration *e.g.* by an inhaler, using a fine spray. The composition may be prepared for nasal, aural or ocular administration *e.g.* as spray or drops. Injectables for intramuscular administration are most typical.

Compositions of the invention may include an antimicrobial, particularly when packaged in multiple dose format. Antimicrobials such as thiomersal and 2-phenoxyethanol are commonly found in vaccines, but it is preferred to use either a mercury-free preservative or no preservative at all.

Compositions of the invention may comprise detergent *e.g.* a polysorbate (Tween), such as polysorbate 80. Detergents are generally present at low levels *e.g.* <0.01%, but higher levels have been suggested for stabilising antigen formulations [106] *e.g.* up to 10%. An example composition may include from 0.01 to 0.05% polysorbate, and this is particularly useful when using lipidated fHbp antigen(s).

Methods of treatment

The invention also provides a method for raising an immune response in a mammal, comprising administering a composition of the invention to the mammal. The immune response is preferably protective against meningococcus and preferably involves antibodies. The method may raise a booster response in a patient that has already been primed.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

The invention also provides compositions of the invention for use as a medicament. The medicament is preferably used, as described above, to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of at least two fHbp antigens, as defined above, in the manufacture of a medicament for raising an immune response, as described above, in a mammal.

These uses and methods are preferably for the prevention and/or treatment of a disease caused by *N.meningitidis e.g.* bacterial (or, more specifically, meningococcal) meningitis, or septicemia.

One way of checking efficacy of therapeutic treatment involves monitoring meningococcal infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against antigens after administration of the composition. Immunogenicity of compositions of the invention can be determined by administering them to test subjects (*e.g.* children 12-16 months age, or animal models) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT) for meningococcus. These immune responses will generally be determined around 4 weeks after administration of the composition, and compared to values determined before administration of the composition. A SBA increase of at least 4-fold or 8-fold is preferred. Where more than one dose of the composition is administered, more than one post-administration determination may be made.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by any other suitable route. The invention may be used to elicit systemic and/or mucosal immunity. Intramuscular administration to the thigh or the upper arm is preferred. Injection may be via a needle (*e.g.* a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dose is 0.5 ml.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Suitable timing between priming doses (*e.g.* between 4-16 weeks), and between priming and boosting, can be routinely determined.

General

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, references 107-113, *etc.*

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

Where the invention concerns an "epitope", this epitope may be a B-cell epitope and/or a T-cell epitope, but will usually be a B-cell epitope. Such epitopes can be identified empirically (*e.g.* using PEPSCAN [114,115] or similar methods), or they can be predicted (*e.g.* using the Jameson-Wolf antigenic index [116], matrix-based approaches [117], MAPITOPE [118], TEPITOPE [119,120], neural networks [121], OptiMer & EpiMer [122,123], ADEPT [124], Tsites [125], hydrophilicity [126], antigenic index [127] or the methods disclosed in references 128-132, *etc.*). Epitopes are the parts of an antigen that are recognised by and bind to the antigen binding sites of antibodies or T-cell receptors, and they may also be referred to as "antigenic determinants".

Where the invention uses a "purified" antigen, this antigen is separated from its naturally occurring environment. For example, the antigen will be substantially free from other meningococcal components, other than from any other purified antigens that are present. A mixture of purified antigens will typically be prepared by purifying each antigen separately and then re-combining them, even if the two antigens are naturally present in admixture.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of ref. 133. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in ref. 134.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

"GI" numbering is used above. A GI number, or "GenInfo Identifier", is a series of digits assigned consecutively to each sequence record processed by NCBI when sequences are added to its databases. The GI number bears no resemblance to the accession number of the sequence record. When a sequence is updated (*e.g.* for correction, or to add more annotation or information) then it receives a new GI number. Thus the sequence associated with a given GI number is never changed.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows adsorption of fHbp antigens to aluminium hydroxide (AH) or aluminium phosphate (AP) adjuvants. Lanes marked "D" are antigen after desorption treatment, whereas "T" lanes are the solution-phase antigens after adsorption. Thus a band in "D" but absent from "T" shows that the antigen is fully adsorbed to the adjuvant, but could be desorbed. The 3 lanes to the left of "D" are an antigen standard at 10%, 5% or 2.5% of the vaccine dose.

Figure 2 is like Figure 1, but shows adsorption of fHbp mixtures.

MODES FOR CARRYING OUT THE INVENTION

Three fHbp proteins were used, representing the following strains and fHbp families:

Strain	MC58	M01573	M01240320
fHbp family	I	I	III
SEQ ID NO	1 / 26	4 / 23	6 / 25

These were tested as monovalent vaccines or as bivalent vaccines (either as MC58+M01573 or MC58+ M01240320).

The antigens were adsorbed to an aluminium hydroxide adjuvant (AH) or to an aluminium phosphate adjuvant (AP). Figure 1 confirms that the individual fHbp antigens adsorbed well to both adjuvants, and could be desorbed intact. Figure 2 shows that this result was also seen with the bivalent mixtures.

These monovalent and bivalent vaccines were also used to immunise mice and the resulting sera were tested for bactericidal activity against various meningococcal strains. The following table shows results against 2 strains from each of fHbp families I, II and III, where a "+" indicates a bactericidal titre of 128 or more:

Strain name (fHbp family)	MC58		M01573		M01240320		MC58 + M01573		MC58 + M01240320	
	AH	AP	AH	AP	AH	AP	AH	AP	AH	AP
MC58 (I)	+	+	-	-	-	-	+	+	+	+
NZ98/254 (I)	-	-	-	-	-	-	+	+	+	-
961-5945 (II)	-	-	-	-	+	-	-	-	+	+
M12566 (II)	-	-	-	-	+	-	-	-	+	+
M01240355 (III)	-	-	-	-	+	+	-	-	+	+
M01240320 (III)	-	-	-	-	+	+	-	-	+	+

Therefore the MC58 antigen was able to protect against the homologous strain with both adjuvants, but not against the other strains. The M01240320 antigen was able to protect against the homologous strain with both adjuvants, and also against another family III strain, but did not protect against family I strains and could protect against family II strains when using an AH adjuvant. The M01573 antigen alone did not protect against any of these six strains, with either adjuvant. Thus results were mixed with the monovalent vaccines.

The bivalent vaccine based on a combination of MC58 and M01573 protected against both family I strains, even though neither of the antigens alone had protected against NZ98/254. This result was seen with both adjuvants. As expected, however, the mixture of two family I antigens did not afford protection against family II or family III strains.

The bivalent vaccine based on a combination of MC58 and M01240320 protected against all six strains (except for one strain when using the AP adjuvant), including family II strains.

Many circulating meningococcal strains are in fHbp family I, and a large proportion of these are in sub-family I.1 (represented by MC58). Thus the M01573 sequence alone is unsuitable for ensuring coverage of this large sub-family. Instead of relying only on M01573, therefore, a vaccine should either replace it, or supplement it with a second family I sequence *e.g.* M01573+MC58. A combination of a sub-family I.1 sequence with a family III sequence can give broad protection using either an AH or an AP adjuvant.

Alignment of SEQ ID NOs: 23 and 26

SEQ ID NOs: 23 and 26 are mature amino acid sequences (N-terminal cysteine) from strains MC58 and M01573, respectively. They align as follows:

```
SEQID_26        CSSGGGG-----VAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEK 55
SEQID_23        CSSGGGGSGGGGVTADIGTGLADALTAPLDHKDKGLKSLTLEDSISQNGTLTLSAQGAEK 60
                *******     *:****:*******************:****::*: :* .*.*:******

SEQID_26        TYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQ 115
SEQID_23        TYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQEQ 120
                ************************************************:**** *

SEQID_26        DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAK 175
SEQID_23        DPEHSEKMVAKRRFRIGDIAGEHTSFDKLPKDVMATYRGTAFGSDDAGGKLTYTIDFAAK 180
                *.*** ******:******************:.  **************************

SEQID_26        QGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVA 235
SEQID_23        QGHGKIEHLKSPELNVDLAVAYIKPDEKHHAVISGSVLYNQDEKGSYSLGIFGEKAQEVA 240
                **:*****************.* **** *:************ *********** ******

SEQID_26        GSAEVKTVNGIRHIGLAAKQ 255
SEQID_23        GSAEVETANGIHHIGLAAKQ 260
                *****:*.***:********
```

Starting from Val-8 of SEQ ID NO: 26 and Val-13 of SEQ ID NO: 23 (*i.e.* excluding the N-terminus repeat region), they are 87.5% identical (217/248 identical residues).

When aligned with sequences from MC58, 961-5945 and M1239, two residues are unique to SEQ ID NO: 23, namely Thr-14 and Val-153, which align with residues 2 and 141 of SEQ ID NO: 1.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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CLAIMS

1. An immunogenic composition which comprises a first fHbp antigen and a second fHbp antigen, present as separate polypeptides, and an aluminium phosphate adjuvant, wherein: the first fHbp antigen comprises an amino acid sequence which has at least 75% sequence identity to both of SEQ ID NO: 26 and SEQ ID NO: 23, but has a higher sequence identity to SEQ ID NO: 26 than to SEQ ID NO: 23, when aligned using the same algorithm and parameters; and the second fHbp antigen comprises an amino acid sequence (i) having at least 94% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least 50 contiguous amino acids from SEQ ID NO: 25.
2. The composition of claim 1, wherein the first fHbp antigen (a) can elicit antibodies in a mouse which are bactericidal against strain MC58 in a serum bactericidal assay.
3. The composition of claim 1 or claim 2, wherein the second fHbp antigen can elicit antibodies in a mouse which are bactericidal against strain 961-5945 in a serum bactericidal assay.
4. The composition of claim 1, or claim 2, wherein the composition does not include (i) an antigen comprising an amino acid sequence which has >95% sequence identity to SEQ ID NO: 23 or (ii) an antigen comprising an amino acid sequence which has >95% sequence identity to SEQ ID NO: 24.
5. The composition of any preceding claim, wherein at least one of the fHbp antigens is lipidated at a N-terminus cysteine.
6. An immunogenic composition comprising a polypeptide and an aluminium phosphate adjuvant, wherein the polypeptide has formula NH_2-A-{-X-L-}$_n$-B-COOH in which:
   - n is 3; a first X is an amino acid sequence which has at least 75% sequence identity to both of SEQ ID NO: 26 and SEQ ID NO: 23, but has a higher sequence identity to SEQ ID NO: 26 than to SEQ ID NO: 23, when aligned using the same algorithm and parameters; a second X is an amino acid sequence which has at least 75% sequence identity to both of SEQ ID NO: 26 and SEQ ID NO: 23, but has a higher sequence identity to SEQ ID NO: 26 than to SEQ ID NO: 23, when aligned using the same algorithm and parameters; a third X is an amino acid sequence (i) having at least 94% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least 50 contiguous amino acids from SEQ ID NO: 25; each L is an optional linker amino acid sequence; A is an optional N terminal amino acid sequence; and B is an optional C terminal amino acid sequence; or
   - n is 2; a first X is an amino acid sequence which has at least 75% sequence identity to both of SEQ ID NO: 26 and SEQ ID NO: 23, but has a higher sequence identity to SEQ ID NO: 26 than to SEQ ID NO: 23, when aligned using the same algorithm and parameters; a second X is an amino acid sequence (i) having at least 94% sequence

CLAIMS

1. An immunogenic composition which comprises a first fHbp antigen and a second fHbp antigen, and an aluminium phosphate adjuvant, wherein: the first fHbp antigen comprises an amino acid sequence which has at least 75% sequence identity to both of SEQ ID NO: 26 and SEQ ID NO: 23, but has a higher sequence identity to SEQ ID NO: 26 than to SEQ ID NO: 23, when aligned using the same algorithm and parameters; and the second fHbp antigen comprises an amino acid sequence (i) having at least 94% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least 50 contiguous amino acids from SEQ ID NO: 25.

2. The composition of claim 1, wherein the first fHbp antigen (a) can elicit antibodies in a mouse which are bactericidal against strain MC58 in a serum bactericidal assay.

3. The composition of claim 1 or claim 2, wherein the second fHbp antigen can elicit antibodies in a mouse which are bactericidal against strain 961-5945 in a serum bactericidal assay.

4. The composition of claim 1, or claim 2, wherein the composition does not include (i) an antigen comprising an amino acid sequence which has >95% sequence identity to SEQ ID NO: 23 or (ii) an antigen comprising an amino acid sequence which has >95% sequence identity to SEQ ID NO: 24.

5. The composition of any preceding claim, wherein at least one of the fHbp antigens is lipidated at a N-terminus cysteine.

6. An immunogenic composition comprising a polypeptide and an aluminium phosphate adjuvant, wherein the polypeptide has formula NH_2-A-{-X-L-}$_n$-B-COOH in which:

- n is 2; a first X is an amino acid sequence which is more closely related to SEQ ID NO: 26 than to SEQ ID NO: 23; a second X is an amino acid sequence which is more closely related to SEQ ID NO: 23 than to SEQ ID NO: 26; each L is an optional linker amino acid sequence; A is an optional N terminal amino acid sequence; and B is an optional C terminal amino acid sequence; or
- n is 3; a first X is an amino acid sequence which is more closely related to SEQ ID NO: 26 than to SEQ ID NO: 23; a second X is an amino acid sequence which is more closely related to SEQ ID NO: 23 than to SEQ ID NO: 26; a third X is an amino acid sequence (i) having at least 94% sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least 50 contiguous amino acids from SEQ ID NO: 25; each L is an optional linker amino acid sequence; A is an optional N terminal amino acid sequence; and B is an optional C terminal amino acid sequence; or
- n is 2; a first X is an amino acid sequence which is more closely related to SEQ ID NO: 26 than to SEQ ID NO: 23; a second X is an amino acid sequence (i) having at least 94%

sequence identity to SEQ ID NO: 25 and/or (ii) consisting of a fragment of at least 50 contiguous amino acids from SEQ ID NO: 25; each L is an optional linker amino acid sequence; A is an optional N terminal amino acid sequence; and B is an optional C terminal amino acid sequence.

7. The composition of any preceding claim, further including a capsular saccharide from meningococcal serogroup(s) A, C, W135 and/or Y, conjugated to a carrier protein.

8. The composition of any preceding claim, further including a pneumococcal capsular saccharide conjugated to a carrier protein.

FIGURE 1

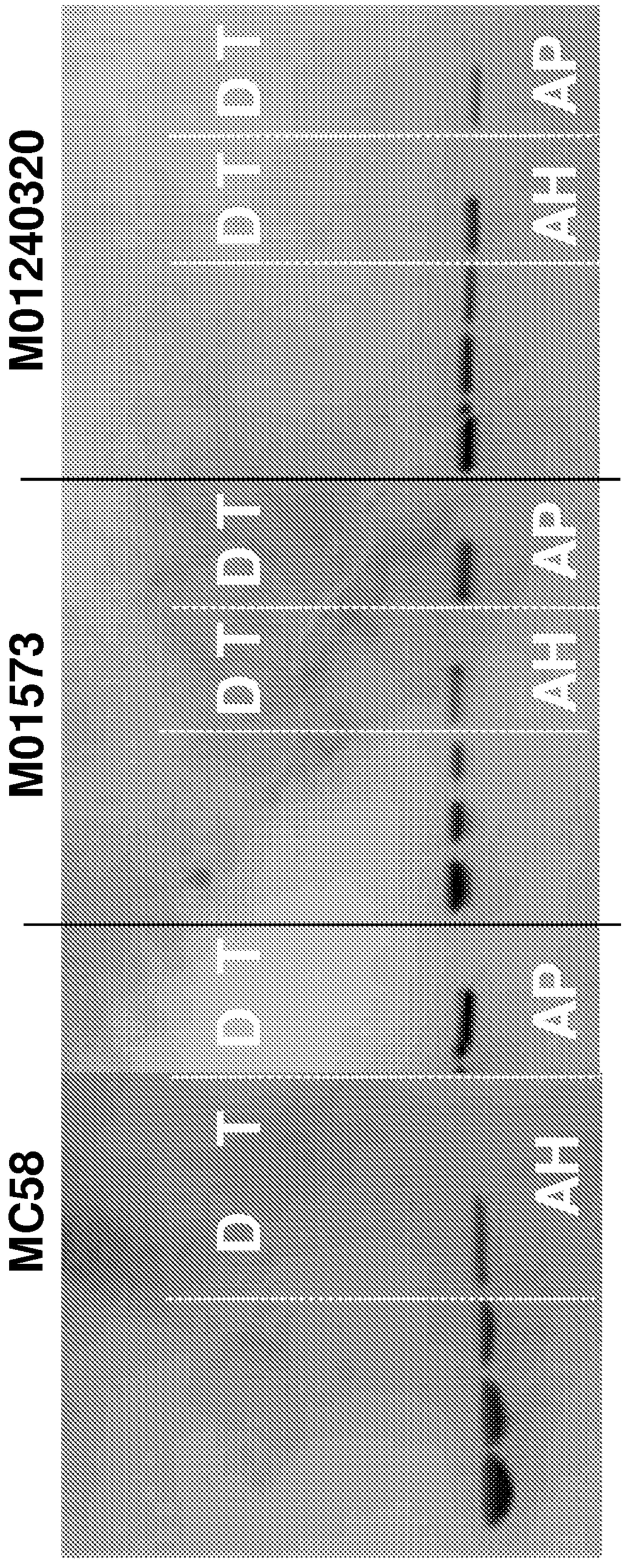

FIGURE 2