(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 September 2010 (30.09.2010)

PCT

(10) International Publication Number
WO 2010/109325 A2

(51) International Patent Classification:
A61K 39/02 (2006.01)

(21) International Application Number:
PCT/IB2010/000735

(22) International Filing Date:
24 March 2010 (24.03.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/162,996 24 March 2009 (24.03.2009) US

(71) Applicant *(for all designated States except US)*: **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **COSTANTINO, Paolo** [IT/IT]; Novartis Vaccines, Via Fiorentina, 1, I-53100 Siena (IT).

(74) Agents: **MARSHALL, Cameron, John** et al.; Carpmaels & Ransford, One Southampton Row, London WC1B 5HA (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

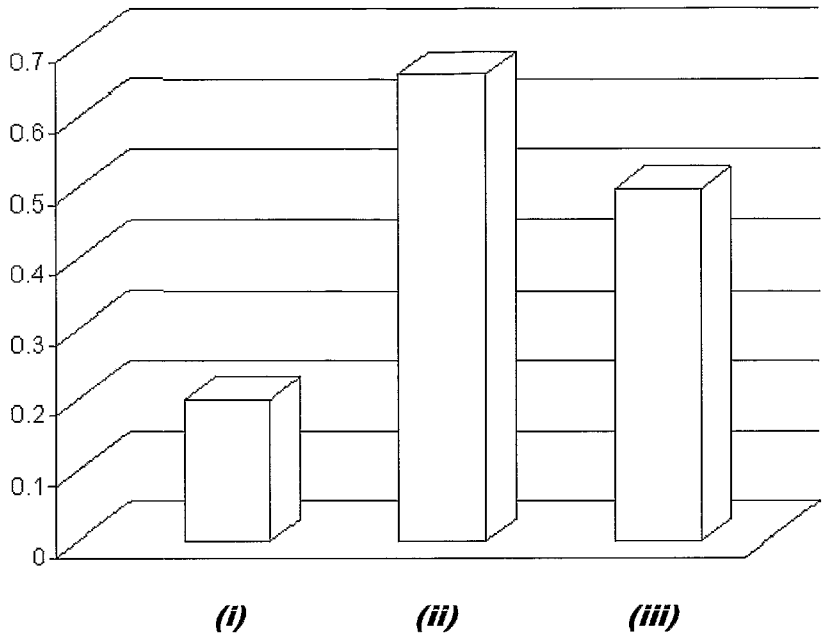
Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: COMBINATIONS INCLUDING PNEUMOCOCCAL SEROTYPE 14 SACCHARIDE

FIGURE 3



(57) Abstract: Meningococcal lipooligosaccharide and pneumococcal serotype 14 capsular saccharide share an antigen that can cross-react with human tissue. The invention provides various ways of minimising the production of autoreactive antibodies when these two antigens are co-administered.

WO 2010/109325 A2

COMBINATIONS INCLUDING PNEUMOCOCCAL SEROTYPE 14 SACCHARIDE

This application claims priority from US provisional application 61/162,996 (filed 24th March 2009), the complete contents of which are hereby incorporated herein by reference.

TECHNICAL FIELD

This invention is in the field of combination vaccines, in particular those containing both a pneumococcal serotype 14 capsular saccharide and a lipooligosaccharide component (*e.g.* from *Neisseria meningitidis*).

BACKGROUND ART

Streptococcus pneumoniae, also known as pneumococcus, is a Gram-positive spherical bacterium. Current pneumococcal vaccines are based on capsular saccharides. The authorised pediatric vaccines are (a) PREVNAR™, which is a 7-valent mixture of conjugated saccharides from serotypes 4, 6B, 9V, 14, 18C, 19F & 23F, (b) SYNFLORIX™, a 10-valent conjugate mixture which also covers serotypes 1, 5 and 7F, and (c) PREVNAR 13™, a 13-valent conjugate mixture which also covers serotypes 3, 6A & 19A. Other 9-, 10-, 11- and 13-valent conjugate combinations are also known.

Neisseria meningitidis, also known as meningococcus, is a Gram-negative spherical bacterium. Current meningococcal vaccines are also based on capsular saccharides. These include monovalent serogroup C conjugate vaccines (MENJUGATE™, MENINGITEC™ and NEISVAC-C™) and 4-valent conjugate mixtures for serogroups A, C, W135 and Y (MENACTRA™). There is currently no useful vaccine authorised for general use against serogroup B ('MenB'). Current research efforts for making a MenB vaccine are focusing on outer membrane vesicles (*e.g.* MENZB™, HEXAMEN™, NONAMEN™) or on purified components from the outer membrane, such as lipooligosaccharide and outer membrane proteins. MenB vaccines based on outer membrane vesicles (OMVs) have received much attention in recent years. For instance, each of Novartis Vaccines, GlaxoSmithKline and RIVM/NVI has a vesicle-based product.

Reference 1 discloses a composition for immunising against both pneumococcus and MenB, formed by combining a 13-valent pneumococcal conjugate vaccine ('13vPnC'; Wyeth's PREVNAR 13™) with a 9-valent MenB outer membrane vesicle vaccine (NONAMEN™, NVI).

There remains a need for further and improved combination vaccines for protecting against both meningococcus serogroup B and pneumococcus.

DISCLOSURE OF THE INVENTION

The inventor has identified a problem with the combination vaccine of reference 1. The 13vPnC component includes pneumococcal serotype 14 capsular saccharide (CS14), and the vesicles in the MenB component include the lipooligosaccharide (LOS; also referred to as LPS) component of the bacterial outer membrane. The inventor has realised that LOS (at least for meningococcal immunotypes L2, L3, L4 and L7) and CS14 share a tetrasaccharide structure Galβ1-4GlcNAcβ1-3Galβ1-4Glc. This tetrasaccharide is known as lacto-N-neotetraose (LNnT) and is also present in

humans, in particular in breast milk and also as the terminal part of lacto-N-neotetraosyl ceramide (also known as paragloboside), which is a biosynthetic precursor of the ABH and P_1 blood group glycosphingolipids and of some gangliosides. Although the LNnT epitope is normally masked *in vivo* in human tissue by terminal sialylation (also seen in MenB, but not in CS14), under some circumstances (*e.g.* in reduced temperatures) it becomes immuno-accessible and antibody binding to the epitope leads to hemolysis. This accessibility leads to an autoimmune reaction known as cold agglutinin disease or AIHA (autoimmune hemolytic anemia).

The inventor has realised that the presence of the LNnT structure in both MenB LOS and CS14 means that administration of either of these antigens to a patient can potentially result in increased levels of anti-LNnT antibodies. If these two antigens are co-administered then there is a risk that very high levels of anti-LNnT antibodies will be elicited, particularly if the antigens are administered with a vaccine adjuvant. Thus a combination vaccine including both MenB LOS and CS14 may present a higher risk of causing AIHA in patients, particularly if the vaccine is administered during winter. The invention aims to decrease this risk by limiting the immunogenic effect of LNnT epitopes in such vaccines, and provides various ways of minimising the risk of producing autoreactive antibodies when MenB and pneumococcal antigens are co-administered.

In a first aspect, the LNnT epitope is disrupted within either or both of the LOS or CS14. Thus the invention provides an immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal serotype 14 capsular saccharide (CS14), wherein the LOS and/or the CS14 do(es) not include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide. The immunogenic composition will typically further include an adjuvant. Where the epitope is present in only one of LOS and CS14, it is preferably absent from LOS.

In a second aspect, the LNnT epitope is retained in both of the LOS and CS14, but the vaccine is unadjuvanted. Thus the invention provides an unadjuvanted immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal serotype 14 capsular saccharide (CS14), wherein the LOS and the CS14 both include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide. For example, the LOS can be from meningococcal immunotype L2 or L3 (or a mixture of both may be included).

In a third aspect, the LNnT epitope is retained in both of the LOS and CS14, but the doses are reduced to low levels. Thus the invention provides an immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal serotype 14 capsular saccharide (CS14), wherein (i) the LOS and the CS14 both include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide, (ii) the concentration of LOS is less than 5μg/ml, and (iii) the concentration of CS14 is less than 5μg/ml.

In a fourth aspect, a vaccine is made against both MenB and type 14 pneumococcus by using a LNnT-containing LOS against MenB but a protein antigen against pneumococcus. Thus the

invention provides an immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal polypeptide antigen, wherein (i) the LOS includes a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide, (ii) the pneumococcal polypeptide can elicit an immune response that is effective against serotype 14 pneumococcus, and (iii) the composition does not include a pneumococcal serotype 14 capsular saccharide.

In a fifth aspect, a vaccine is made against both MenB and type 14 pneumococcus by using a LNnT-containing CS14 capsular saccharide but a non-LOS antigen against MenB. Thus the invention provides an immunogenic composition comprising a meningococcal polypeptide and a pneumococcal serotype 14 capsular saccharide, wherein (i) the pneumococcal serotype 14 capsular saccharide includes a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide, (ii) the meningococcal polypeptide can elicit an immune response that is effective against serogroup B meningococcus, and (iii) the composition does not include a meningococcal lipooligosaccharide.

In some embodiments of the invention, immunogenic compositions include no more than one meningococcal PorA serosubtype *e.g.* they may include no PorA outer membrane protein.

Meningococcal lipooligosaccharide

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). Figure 1 shows the core saccharides for a L3 immunotype. The α-chain linked to Hep^{I} (heptose) contains the LNnT tetrasaccharide with a sialic acid cap. The same structure is seen in immunotypes L7 and L9. The L2 and L4 immunotypes include the same α chain as L3 but have different β- and/or γ-chains attached to Hep^{II}. The KDO^{I} residue (2-keto-3-deoxy-octulosonic acid) is attached to the lipid A portion of LOS and in often also linked to a second KDO residue (shown as KDO^{II} in Figure 1).

The L2 and L3 α-chains include a LNnT tetrasaccharide. Where the invention uses a LOS that does not include a LNnT tetrasaccharide then it may use LOS from a different immunotype (*e.g.* from L1, L4, L5, L6 or L8). In some embodiments, however, it may be desirable to retain L2 and/or L3 epitopes (other than their LNnT epitopes). This 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 (*e.g.* see references 2 to 7). 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, if the strain in question has a *lgtC* gene (*e.g.* the MC58 strain of serogroup B

meningococcus, with L3 immunotype, consists of *lgtA, lgtB* and *lgtE*, without *lgtC* and *lgtD*). 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.

Beyond 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 [8]. At least one primary O-linked fatty acid may be removed from LOS [9]. LOS having a reduced number of secondary acyl chains per LOS molecule can also be used [10]. The LOS will typically include at least the GlcNAc-Hep_2phosphoethanolamine-KDO_2-Lipid A structure [11]. 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. It may be present within meningococcal outer membrane vesicles. It may be conjugated to meningococcal outer membrane vesicles.

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 9, 11, 12, 13, *etc.* Preferred carrier proteins for these conjugates are bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof. These are commonly used in conjugate vaccines. The CRM_{197} diphtheria toxin mutant is useful [14]. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein complex [15], synthetic peptides [16,17], heat shock proteins [18,19], pertussis proteins [20,21], cytokines [22], lymphokines [22], hormones [22], growth factors [22], artificial proteins comprising multiple human $CD4^+$ T cell epitopes from various pathogen-derived antigens [23] such as N19 [24], protein D from *H.influenzae* [25-27], pneumolysin [28] or its non-toxic derivatives [29], pneumococcal surface protein PspA [30], iron-uptake proteins [31], toxin A or B from *C.difficile* [32], recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) [33], *etc.*

LOS can be present within a vesicle. Such vesicles include any proteoliposomic vesicle obtained by disrupting or blebbing from a meningococcal outer membrane to form vesicles therefrom that include protein components and LOS from the outer membrane. Thus the term includes OMVs (sometimes referred to as 'blebs'), microvesicles (MVs [34]) and 'native OMVs' ('NOMVs' [35]).

MVs and NOMVs are naturally-occurring membrane vesicles that form spontaneously during bacterial growth and are released into culture medium. MVs can be obtained by culturing *Neisseria* in broth culture medium, separating whole cells from the smaller MVs in the broth culture medium (*e.g.* by filtration or by low-speed centrifugation to pellet only the cells and not the smaller vesicles), and then collecting the MVs from the cell-depleted medium (*e.g.* by filtration, by differential

precipitation or aggregation of MVs, by high-speed centrifugation to pellet the MVs). Strains for use in production of MVs can generally be selected on the basis of the amount of MVs produced in culture *e.g.* refs. 36 & 37 describe *Neisseria* with high MV production.

OMVs are prepared artificially from bacteria, and may be prepared using detergent treatment (*e.g.* with deoxycholate), or by non-detergent means (*e.g.* see reference 38). Techniques for forming OMVs include treating bacteria with a bile acid salt detergent (*e.g.* salts of lithocholic acid, chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid, cholic acid, ursocholic acid, *etc.*, with sodium deoxycholate [39 & 40] being preferred for treating *Neisseria*) at a pH sufficiently high not to precipitate the detergent [41]. Other techniques may be performed substantially in the absence of detergent [38] using techniques such as sonication, homogenisation, microfluidisation, cavitation, osmotic shock, grinding, French press, blending, *etc.* Methods using no or low detergent can retain useful antigens such as NspA [38]. Thus a method may use an OMV extraction buffer with about 0.5% deoxycholate or lower *e.g.* about 0.2%, about 0.1%, <0.05% or zero.

A useful process for OMV preparation is described in reference 42 and involves ultrafiltration on crude OMVs, rather than instead of high speed centrifugation. The process may involve a step of ultracentrifugation after the ultrafiltration takes place.

Vesicles for use with the invention can be prepared from any meningococcal strain. The vesicles will usually be from a serogroup B strain, but it is possible to prepare them from serogroups other than B (*e.g.* reference 41 discloses a process for serogroup A), such as A, C, W135 or Y. The strain may be of any serotype (*e.g.* 1, 2a, 2b, 4, 14, 15, 16, *etc.*), any serosubtype, and any immunotype (*e.g.* L1; L2; L3; L3,3,7; L10; *etc.*). The meningococci may be from any suitable lineage, including hyperinvasive and hypervirulent lineages *e.g.* any of the following seven hypervirulent lineages: subgroup I; subgroup III; subgroup IV-1; ET-5 complex; ET-37 complex; A4 cluster; lineage 3. These lineages have been defined by multilocus enzyme electrophoresis (MLEE), but multilocus sequence typing (MLST) has also been used to classify meningococci [ref. 43] *e.g.* the ET-37 complex is the ST-11 complex by MLST, the ET-5 complex is ST-32 (ET-5), lineage 3 is ST-41/44, *etc.* Vesicles can be prepared from strains having one of the following subtypes: P1.2; P1.2,5; P1.4; P1.5; P1.5,2; P1.5,c; P1.5c,10; P1.7,16; P1.7,16b; P1.7h,4; P1.9; P1.15; P1.9,15; P1.12,13; P1.13; P1.14; P1.21,16; P1.22,14.

Vesicles used with the invention may be prepared from wild-type meningococcal strains or from mutant meningococcal strains. For instance, reference 44 discloses preparations of vesicles obtained from *N.meningitidis* with a modified *fur* gene. Reference 51 teaches that *nspA* expression should be up-regulated with concomitant *porA* and *cps* knockout. Further knockout mutants of *N.meningitidis* for OMV production are disclosed in references 3, 51 and 52. Reference 45 discloses vesicles in which fHBP is upregulated. Reference 46 discloses the construction of vesicles from strains modified to express six different PorA subtypes. These or others mutants can all be used with the invention.

Thus a strain used with the invention may in some embodiments express more than one PorA subtype. 6-valent and 9-valent PorA strains have previously been constructed. The strain may express 2, 3, 4, 5, 6, 7, 8 or 9 of PorA subtypes: P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1 and/or P1.18-1,3,6. In other embodiments, however, a strain may have been down-regulated for PorA expression *e.g.* in which the amount of PorA has been reduced by at least 20% (*e.g.* ≥30%, ≥40%, ≥50%, ≥60%, ≥70%, ≥80%, ≥90%, ≥95%, *etc.*), or even knocked out, relative to wild-type levels (*e.g.* relative to strain H44/76, as disclosed in ref. 51).

Advantageously, vesicles prepared from a $LgtB^{-ve}$ strain of meningococcus have been shown to improve the anti-CS14 response in a combined preparation.

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 47. 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 47 discloses how this result can be achieved by modification of the *lgtA* and/or *lgtG* gene products.

In some embodiments a strain may hyper-express (relative to the corresponding wild-type strain) certain proteins. For instance, strains may hyper-express NspA, protein 287 [48], fHBP [45], TbpA and/or TbpB [49], Cu,Zn-superoxide dismutase [49], *etc.*

In some embodiments a strain may include one or more of the knockout and/or hyper-expression mutations disclosed in references 3 and 50 to 52. Useful genes for down-regulation and/or knockout include: (a) Cps, CtrA, CtrB, CtrC, CtrD, FrpB, GalE, HtrB/MsbB, LbpA, LbpB, LpxK, Opa, Opc, PilC, PorB, SiaA, SiaB, SiaC, SiaD, TbpA, and/or TbpB [50]; (b) CtrA, CtrB, CtrC, CtrD, FrpB, GalE, HtrB/MsbB, LbpA, LbpB, LpxK, Opa, Opc, PhoP, PilC, PmrE, PmrF, SiaA, SiaB, SiaC, SiaD, TbpA, and/or TbpB [51]; (c) ExbB, ExbD, rmpM, CtrA, CtrB, CtrD, GalE, LbpA, LpbB, Opa, Opc, PilC, PorB, SiaA, SiaB, SiaC, SiaD, TbpA, and/or TbpB [52]; and (d) CtrA, CtrB, CtrD, FrpB, OpA, OpC, PilC, PorB, SiaD, SynA, SynB, and/or SynC [3].

Where a mutant strain is used, in some embodiments it may have one or more, or all, of the following characteristics, optionally in addition to LNnT-disrupting mutation(s): (i) up-regulated TbpA; (ii) up-regulated NhhA; (iii) up-regulated Omp85; (iv) up-regulated LbpA; (v) up-regulated NspA; (vi) knocked-out PorA; (vii) down-regulated or knocked-out FrpB; (viii) down-regulated or knocked-out Opa; (ix) down-regulated or knocked-out Opc; (x) deleted *cps* gene complex.

Advantageously, LOS in a vesicle can be treated so as to link the LOS and protein components in the vesicle ("intra-bleb" conjugation [3]).

LOS may be O-acetylated on a GlcNac residue attached to its Heptose II residue *e.g.* for L3 [53].

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 54 may be used.

In some embodiments, LOS is present in a composition at less than 5μg/ml *e.g.* ≤4μg/ml, ≤3μg/ml, ≤2μg/ml, ≤1μg/ml. This low concentration can be useful when CS14 retains a LNnT epitope.

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

Pneumococcal serotype 14 capsular saccharide

Compositions of the invention include a pneumococcal serotype 14 capsular saccharide (CS14). Reference 55 reports that CS14 includes the repeating structure shown in Figure 2.

CS14 saccharide used with the invention will usually include wild-type repeating units but, in some embodiments, may be modified not to include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide. This modification can be achieved by knockout of one or more of the relevant biosynthetic enzymes or by chemical and/or enzymatic treatment of the saccharide to modify one or more of the four residues within the tetrasaccharide. For instance, endo-β-galactosidase, purified from culture supernatants of *Cytophaga keratolytica*, catalyzes the hydrolysis of galactose β(1→4) glucose bonds of susceptible polysaccharides including CS14 [55].

The CS14 may be N-acetylated. As described in reference 56, for instance, it may be more than 50%, 60%, 70%, 80%, or 90% N-acetylated.

The CS14 will typically be included in a composition as a conjugate. Suitable carrier proteins for such conjugates are described above *e.g.* bacterial toxins, such as diphtheria or tetanus toxins, or toxoids or mutants thereof, such as CRM_{197}, the *N.meningitidis* outer membrane protein complex, synthetic peptides, heat shock proteins, pertussis proteins, cytokines, lymphokines, hormones, growth factors, artificial proteins comprising multiple human $CD4^+$ T cell epitopes from various pathogen-derived antigens such as N19, protein D from *H.influenzae*, pneumolysin or its non-toxic derivatives, pneumococcal surface protein PspA, iron-uptake proteins, toxin A or B from *C.difficile*, rEPA, *etc.* Particularly useful carrier proteins for CS14 are CRM197, tetanus toxoid, diphtheria toxoid and *H.influenzae* protein D. CRM197, as seen in PREVNAR™, is very useful.

The carrier molecule may be covalently conjugated to the CS14 directly or via a linker. Various linkers are known *e.g.* an adipic acid linker, 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 [57,58]. Another preferred type of linkage is a carbonyl linker, which may be formed by reaction of a free hydroxyl group of a modified saccharide with CDI [59, 60] followed by reaction with a protein to form a carbamate linkage. Other linkers include β-propionamido [61], nitrophenyl-ethylamine [62], haloacyl halides [63], glycosidic linkages [64], 6-aminocaproic acid [65], N-succinimidyl-3-(2-

pyridyldithio)-propionate (SPDP) [66], adipic acid dihydrazide ADH [67], C_4 to C_{12} moieties [68], *etc.* Carbodiimide condensation can also be used [69].

Conjugation of CS14 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.

The CS14 saccharide may comprise a full-length intact saccharide as prepared from pneumococcus, 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. Intact CS14, as seen in Prevnar™, is preferred.

In some embodiments, CS14 is present in a composition at less than 5μg/ml *e.g.* ≤4μg/ml, ≤3μg/ml, ≤2μg/ml, ≤1μg/ml. This low concentration can be useful when LOS retains a LNnT epitope. A concentration of about 4μg/ml is convenient.

The CS14 antigen can preferably elicit anticapsular antibodies that bind to CS14 *e.g.* elicit an anti-CS14 antibody level ≥0.20μg/mL [70]. 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.

Alternative meningococcal antigens

In some embodiments of the invention, a composition includes a LNnT-containing CS14 capsular saccharide but a non-LOS antigen against MenB. Alternatives to LOS include polypeptide antigens such as fHBP, 287, NadA, NspA, HmbR, NhhA, App, and/or Omp85. These antigens will usefully be present as purified polypeptides *e.g.* recombinant polypeptides.

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

fHBP (factor H binding protein)

A composition of the invention may include a fHBP antigen. The fHBP antigen has been characterised in detail. It has also been known as protein '741' [SEQ IDs 2535 & 2536 in ref. 81], 'NMB1870', 'GNA1870' [refs. 71-73], 'P2086', 'LP2086' or 'ORF2086' [74-76]. 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 [77]. 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 [78] 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. The invention can use a single fHBP variant, but is will usefully include a fHBP from two or three of the variants.

Where the invention uses a single fHBP variant, a composition may include a polypeptide comprising (a) an amino acid sequence having at least *a%* sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 1; or (b) an amino acid sequence having at least *b%* sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least *y* contiguous amino acids from SEQ ID NO: 2; or (c) an amino acid sequence having at least *c%* sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least *z* contiguous amino acids from SEQ ID NO: 3.

Where the invention uses a fHBP from two or three of the variants, a composition may include a combination of two or three different fHBPs selected from: (a) a first polypeptide, comprising an amino acid sequence having at least *a%* sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 1; (b) a second polypeptide, comprising an amino acid sequence having at least *b%* sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least *y* contiguous amino acids from SEQ ID NO: 2; and/or (c) a third polypeptide, comprising an amino acid sequence having at least *c%* sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least *z* contiguous amino acids from SEQ ID NO: 3. The first, second and third polypeptides have different amino acid sequences.

Where the invention uses a fHBP from two of the variants, a composition can include both: (a) a first polypeptide, comprising an amino acid sequence having at least *a%* sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 1; and (b) a second polypeptide, comprising an amino acid sequence having at least *b%* sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least *y* contiguous amino acids from SEQ ID NO: 2. The first and second polypeptides have different amino acid sequences.

Where the invention uses a fHBP from two of the variants, a composition can include both: (a) a first polypeptide, comprising an amino acid sequence having at least *a%* sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least *x* contiguous amino acids from SEQ ID NO: 1; (b) a second polypeptide, comprising an amino acid sequence

having at least *c%* sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least *z* contiguous amino acids from SEQ ID NO: 3. The first and second polypeptides have different amino acid sequences.

The value of *a* is at least 85 *e.g.* 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or more. The value of *b* is at least 85 *e.g.* 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or more. The value of *c* is at least 85 *e.g.* 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 99.5, or more. The values of *a, b* and *c* are not intrinsically related to each other.

The value of *x* is 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, 225, 250). The value of *y* is 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, 225, 250). The value of *z* is 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, 225, 250). The values of *x, y* and *z* are not intrinsically related to each other. Fragments of SEQ ID NOs: 1, 2 or 3 preferably comprise an epitope of the relevant SEQ ID.

A useful composition can include a polypeptide comprising an amino acid sequence having at least 90% (*e.g.* at least 93%) sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least 40 contiguous amino acids from SEQ ID NO: 3.

In some embodiments fHBP polypeptide(s) will be lipidated *e.g.* at a N-terminus cysteine, usually forming tripalmitoyl-S-glyceryl-cysteine. In other embodiments they will not be lipidated.

Administration of a fHBP will preferably elicit antibodies which can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 1, 2 or 3. Advantageous fHBP antigens for use with the invention can elicit bactericidal anti-meningococcal antibodies after administration to a subject.

Where one or more fHBP polypeptide(s) is present, the total dose of fHBP may be between 60 µg/dose and 200µg/dose.

287

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 [79] 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 80, and in example 13 and figure 21 of reference 81 (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.

NadA (Neisserial adhesin A)

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 [79] 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. SEQ ID NO: 6 is one such fragment.

NspA (Neisserial surface protein A)

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 [79] as gene NMB0663 (GenBank accession number GI:7225888; SEQ ID NO: 11 herein). The antigen was previously known from references 82 & 83. 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.

HmbR

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 [79] as gene NMB1668 (SEQ ID NO: 7 herein). Reference 84 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 85.

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.

Unlike reference 31, the HmbR antigen of the invention will normally not be conjugated to a capsular saccharide antigen.

NhhA (Neisseria hia homologue)

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 [79] 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 80 & 86, 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.

App (Adhesion and penetration protein)

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 [79] 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.

Omp85 (85kDa outer membrane protein)

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 [79] 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 87 and 88. 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.

Alternative pneumococcal antigens

Rather than include CS14 in a composition, in some embodiments a vaccine includes a LNnT-containing LOS for protecting against MenB but includes a protein antigen against pneumococcus. The pneumococcal polypeptide can elicit an immune response that is effective against serotype 14 pneumococcus.

A composition may include one or more of: (1) a spr0057 antigen; (2) a spr0286 antigen; (3) a spr0565 antigen; (4) a spr1098 antigen; (5) a spr1345 antigen; (6) a spr1416 antigen; (7) a spr1418 antigen; (8) a spr0867 antigen; (9) a spr1431 antigen; (10) a spr1739 antigen; (11) a spr2021 antigen; (12) a spr0096 antigen; (13) a spr1433 antigen; and/or (14) a spr1707 antigen.

A composition may include one or more of: (1) a PspA polypeptide; (2) a PsaA polypeptide; (3) a PspC polypeptide; (4) a LytA polypeptide; (5) a PhtA polypeptide; (6) a PhtA polypeptide; (7) a PhtA polypeptide; and/or (8) a PhtD polypeptide.

A composition may include a subunit of a pneumococcal pilus, such as RrgA, RrgB and/or RrgC.

The pneumococcal polypeptide antigen can preferably elicit protective antibodies after administration to a subject.

spr0057

The original 'spr0057' sequence was annotated in reference 89 as 'Beta-N-acetyl-hexosaminidase precursor' (see GI:15902101). For reference purposes, the amino acid sequence of full length spr0057 as found in the R6 strain is given as SEQ ID NO: 18 herein.

Preferred spr0057 polypeptides 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: 18; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 18, 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). These spr0057 proteins

include variants of SEQ ID NO: 18. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 18. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 18 while retaining at least one epitope of SEQ ID NO: 18. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 32, which omits the natural leader peptide and sortase recognition sequences.

spr0286

The original 'spr0286' sequence was annotated in reference 89 as 'Hyaluronate lyase precursor' (see GI:15902330). For reference purposes, the amino acid sequence of full length spr0286 as found in the R6 strain is given as SEQ ID NO: 19 herein.

Preferred spr0286 polypeptides 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: 19; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 19, 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). These spr0286 proteins include variants of SEQ ID NO: 19. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 19. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 19 while retaining at least one epitope of SEQ ID NO: 19. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 33, which omits the natural leader peptide and sortase recognition sequences. Other suitable fragments are SEQ ID NOs: 34 and 35.

spr0565

The original 'spr0565' sequence was annotated in reference 89 as 'beta-galactosidase precursor' (see GI:15902609). For reference purposes, the amino acid sequence of full length spr0565 as found in the R6 strain is given as SEQ ID NO: 20 herein.

Preferred spr0565 polypeptides 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: 20; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 20, 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). These spr0565 proteins include variants of SEQ ID NO: 20 (*e.g.* SEQ ID NO: 66; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 20. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 20 while retaining at least one epitope of SEQ ID NO: 20. Other fragments omit one or more protein

domains. One suitable fragment is SEQ ID NO: 36, which omits the natural leader peptide and sortase recognition sequences. Other suitable fragments are SEQ ID NOs: 37 and 38.

A variant form of spr0565 is SEQ ID NO: 39 herein. The use of this variant form for immunisation is reported in reference 90 (SEQ ID NO: 178 therein). Useful spr0565 polypeptides may 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: 39; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 39, 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). These polypeptides include variants of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39 while retaining at least one epitope of SEQ ID NO: 39. Other fragments omit one or more protein domains.

Immunogenic fragments of SEQ ID NO: 39 are identified in table 1 of reference 90.

Because spr0565 is naturally a long polypeptide (>2000 aa) it can be more convenient to express fragments. Thus a suitable form of spr0565 for use with the invention may be less than 1500 amino acids long (*e.g.* <1400, <1300, <1200, <1100, *etc.*). Such short forms of spr0565 include 'spr0565A' (SEQ ID NO: 37) and 'spr0565B' (SEQ ID NO: 38).

spr1098

The original 'spr1098' sequence was annotated in reference 89 as 'Sortase' (see GI:15903141). For reference purposes, the amino acid sequence of full length spr1098 as found in the R6 strain is given as SEQ ID NO: 21 herein.

Preferred spr1098 polypeptides 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: 21; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 21, 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). These spr1098 proteins include variants of SEQ ID NO: 21. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 21. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 21 while retaining at least one epitope of SEQ ID NO: 21. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 40, which omits the natural leader peptide sequence.

spr1345

The original 'spr1345' sequence was annotated in reference 89 as 'hypothetical protein' (see GI:15903388). For reference purposes, the amino acid sequence of full length spr1345 as found in the R6 strain is given as SEQ ID NO: 22 herein.

Preferred spr1345 polypeptides 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: 22; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 22, 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). These spr1345 proteins include variants of SEQ ID NO: 22. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 22. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 22 while retaining at least one epitope of SEQ ID NO: 22. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 41, which omits the natural leader peptide and sortase recognition sequences.

spr1416

The original 'spr1416' sequence was annotated in reference 89 as 'hypothetical protein' (see GI:15903459). For reference purposes, the amino acid sequence of full length spr1416 as found in the R6 strain is given as SEQ ID NO: 23 herein.

Preferred spr1416 polypeptides 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: 23; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 23, 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). These spr1416 proteins include variants of SEQ ID NO: 23. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 23. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 23 while retaining at least one epitope of SEQ ID NO: 23. Other fragments omit one or more protein domains.

spr1418

The original 'spr1418' sequence was annotated in reference 89 as 'hypothetical protein' (see GI:15903461). For reference purposes, the amino acid sequence of full length spr1418 as found in the R6 strain is given as SEQ ID NO: 24 herein.

Preferred spr1418 polypeptides 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: 24; and/or (b) comprising a fragment of

at least 'n' consecutive amino acids of SEQ ID NO: 24, 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). These spr1418 proteins include variants of SEQ ID NO: 24. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 24. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 24 while retaining at least one epitope of SEQ ID NO: 24. Other fragments omit one or more protein domains.

spr0867

The original 'spr0867' sequence was annotated in reference 89 as 'Endo-beta-N-acetylglucosaminidase' (see GI:15902911). For reference purposes, the amino acid sequence of full length spr0867 as found in the R6 strain is given as SEQ ID NO: 25 herein.

Preferred spr0867 polypeptides 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: 25; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 25, 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). These spr0867 proteins include variants of SEQ ID NO: 25. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 25. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 25 while retaining at least one epitope of SEQ ID NO: 25. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 42, which omits the natural leader peptide sequence.

spr1431

The original 'spr1431' sequence was annotated in reference 89 as '1,4-beta-N-acetylmuramidase' (see GI:15903474). It is also known as 'LytC', and its use for immunisation is reported in reference 104. For reference purposes, the amino acid sequence of full length spr1431 as found in the R6 strain is given as SEQ ID NO: 26 herein.

Preferred spr1431 polypeptides 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: 26; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 26, 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). These spr1431 proteins include variants of SEQ ID NO: 26. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 26. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 26 while retaining at least one epitope of

SEQ ID NO: 26. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 43, which omits the natural leader peptide sequence.

spr1739

The 'spr1739' polypeptide is pneumolysin (*e.g.* see GI:15903781). For reference purposes, the amino acid sequence of full length spr1739 as found in the R6 strain is given as SEQ ID NO: 27 herein.

Preferred spr1739 polypeptides 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: 27; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 27, 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). These spr1739 proteins include variants of SEQ ID NO: 27. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 27. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 27 while retaining at least one epitope of SEQ ID NO: 27. Other fragments omit one or more protein domains.

Mutant forms of pneumolysin for vaccination use are known in the art [29, 91-96], and these mutant forms may be used with the invention. Detoxification can be achieved by C-terminal truncation (*e.g.* see ref. 97) *e.g.* deleting 34 amino acids, 45 amino acids, 7 amino acids [98], *etc.* Further mutations, numbered according to SEQ ID NO: 27, include Pro325→Leu (*e.g.* SEQ ID NO: 44) and/or Trp433→Phe (*e.g.* SEQ ID NO: 45). These mutations may be combined with C-terminal truncations *e.g.* to combine a Pro325→Leu mutation with a 7-mer truncation (*e.g.* SEQ ID NO: 46).

spr2021

The original 'spr2021' sequence was annotated in reference 89 as 'General stress protein GSP-781' (see GI:15904062). For reference purposes, the amino acid sequence of full length spr2021 as found in the R6 strain is given as SEQ ID NO: 28 herein.

Preferred spr2021 polypeptides 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: 28; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 28, 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). These spr2021 proteins include variants of SEQ ID NO: 28. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 28. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 28 while retaining at least one epitope of SEQ ID NO: 28. Other fragments omit one or more protein domains. One suitable fragment is SEQ ID NO: 47, which omits the natural leader peptide sequence.

Reference 90 annotates spr2021 as a secreted 45kDa protein with homology to GbpB and discloses its use as an immunogen (SEQ ID NO: 243 therein; SP2216). Immunogenic fragments of spr2021 are identified in table 1 of reference 90 (page 73). Another useful fragment of spr2021 is disclosed as SEQ ID NO: 1 of reference 99 (amino acids 28-278 of SEQ ID NO: 28 herein).

spr0096

The original 'spr0096' sequence was annotated in reference 89 as 'hypothetical protein' (see GI:15902140). For reference purposes, the amino acid sequence of full length spr0096 as found in the R6 strain is given as SEQ ID NO: 29 herein.

Preferred spr0096 polypeptides 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: 29; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 29, 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). These spr0096 proteins include variants of SEQ ID NO: 29 (*e.g.* SEQ ID NO: 40; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 29. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 29 while retaining at least one epitope of SEQ ID NO: 29. Other fragments omit one or more protein domains.

A variant form of spr0096, with an insert near its C-terminus relative to SEQ ID NO: 29, is SEQ ID NO: 48 herein. The use of this variant for immunisation is reported in reference 90 (SEQ ID NO: 150 therein), where it is annotated as a LysM domain protein. Thus a spr0096 for use with the invention may 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: 48; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 48, 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). These polypeptides include variants of SEQ ID NO: 48. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 48. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 48 while retaining at least one epitope of SEQ ID NO: 48. Other fragments omit one or more protein domains. Immunogenic fragments of SEQID NO: 48 are identified in table 1 of reference 90.

A spr0096 polypeptide may be used in the form of a dimer *e.g.* a homodimer.

spr1433

The original 'spr1433' sequence was annotated in reference 89 as 'hypothetical protein' (see GI:15903476). For reference purposes, the amino acid sequence of full length spr1433 as found in the R6 strain is given as SEQ ID NO: 30 herein.

Preferred spr1433 polypeptides 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: 30; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 30, 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). These spr1433 proteins include variants of SEQ ID NO: 30. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 30. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 30 while retaining at least one epitope of SEQ ID NO: 30. Other fragments omit one or more protein domains.

spr1707

The original 'spr1707' sequence was annotated in reference 89 as 'ABC transporter substrate-binding protein - oligopeptide transport' (see GI:15903749). For reference purposes, the amino acid sequence of full length spr1707 as found in the R6 strain is given as SEQ ID NO: 31 herein.

Preferred spr1707 polypeptides 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: 31; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 31, 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). These spr1707 proteins include variants of SEQ ID NO: 31 (*e.g.* SEQ ID NO: 100; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 31. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 31 while retaining at least one epitope of SEQ ID NO: 31. Other fragments omit one or more protein domains.

A variant form of spr1707, differing from SEQ ID NO: 31 by 4 amino acids, is SEQ ID NO: 49 herein. The use of SEQ ID NO: 49for immunisation is reported in reference 90 (SEQ ID NO: 220 therein). Thus a spr1707 polypeptide for use with the invention may 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: 49; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 49, 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). These polypeptides include variants of SEQ ID NO: 49. Preferred fragments of (b) comprise an epitope

from SEQ ID NO: 49. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 49 while retaining at least one epitope of SEQ ID NO: 49. Other fragments omit one or more protein domains.

Immunogenic fragments of SEQ ID NO: 49 are identified in table 1 of reference 90.

PspA

PspA is the Pneumococcal surface protein A. For reference purposes, the amino acid sequence of full length PspA is SEQ ID NO: 50 herein. In the R6 genome PspA is spr0121 [89].

Preferred PspA polypeptides 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: 50; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 50, 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). These PspA proteins include variants of SEQ ID NO: 50. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 50. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 50 while retaining at least one epitope of SEQ ID NO: 50. Other fragments omit one or more protein domains.

The use of PspA for immunisation is reported *inter alia* in reference 100. It can advantageously be administered in combination with PspC.

PsaA

PsaA is the Pneumococcal surface adhesin. For reference purposes, the amino acid sequence of full length PsaA is SEQ ID NO: 51 herein.

Preferred PsaA polypeptides 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: 51; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 51, 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). These PsaA proteins include variants of SEQ ID NO: 51. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 51. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 51 while retaining at least one epitope of SEQ ID NO: 51. Other fragments omit one or more protein domains. A useful fragment of PsaA is disclosed as SEQ ID NO: 3 in reference 99 (corresponding to amino acids 21-519 of SEQ ID NO: 51 herein).

The use of PsaA for immunisation is reported in reference 101. It can be used in combination with PspA and/or PspC.

PspC

PspC is the pneumococcal surface protein C [102] and is also known as choline-binding protein A (CbpA). Its use for immunisation is reported in references 103 and 104. In the R6 strain it is spr1995 and, for reference, the amino acid sequence of full length spr1995 is SEQ ID NO: 52 herein.

Preferred PspC polypeptides 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: 52; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 52, 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). These spr1995 proteins include variants of SEQ ID NO: 52 (*e.g.* SEQ ID NO: 27; see below). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 52. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 52 while retaining at least one epitope of SEQ ID NO: 52. Other fragments omit one or more protein domains.

A variant of PspC is known as 'Hic'. It is similar to PspC, as shown in Figure 1 of reference 105, where it is reported to bind to factor H (fH). For reference purposes, the amino acid sequence of full length Hic is SEQ ID NO: 53 herein. A Hic protein may be used with the invention in addition to or in place of a PspC polypeptide.

Preferred Hic polypeptides 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: 53; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 53, 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). These Hic proteins include variants of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53 while retaining at least one epitope of SEQ ID NO: 53. Other fragments omit one or more protein domains.

PspC and/or Hic can advantageously be used in combination with PspA and/or PsaA.

LytA

LytA is the N-acetylmuramoyl-L-alanine amidase (autolysin). For reference purposes, the amino acid sequence of full length LytA is SEQ ID NO: 54 herein. In the R6 genome LytA is spr1754 [89].

Preferred LytA polypeptides 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: 54; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 54, 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). These LytA proteins include variants of SEQ ID NO: 54 (*e.g.* GI:18568354). Preferred fragments of (b) comprise an epitope from SEQ ID NO: 54. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 54 while retaining at least one epitope of SEQ ID NO: 54. Other fragments omit one or more protein domains.

The use of LytA for immunisation is reported in reference 106, particularly in a form comprising the LytA choline binding domain fused to a heterologous promiscuous T helper epitope.

PhtA

PhtA is the Pneumococcal histidine triad protein A. For reference purposes, the amino acid sequence of full length PhtA precursor is SEQ ID NO: 55 herein. In the R6 genome PhtA is spr1061 [89].

Preferred PhtA polypeptides 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: 55; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 55, 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). These PhtA proteins include variants of SEQ ID NO: 55. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 55. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 55 while retaining at least one epitope of SEQ ID NO: 55. Other fragments omit one or more protein domains.

The use of PhtA for immunisation is reported in references 107 and 108.

PhtB

PhtB is the pneumococcal histidine triad protein B. For reference purposes, the amino acid sequence of full length PhtB precursor is SEQ ID NO: 56 herein. Xaa at residue 578 can be Lysine.

Preferred PhtB polypeptides 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: 56; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 56, 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). These PhtB proteins include variants of SEQ ID NO: 56. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 56. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or

more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 56 while retaining at least one epitope of SEQ ID NO: 56. Other fragments omit one or more protein domains.

The use of PhtB for immunisation is reported in references 107, 108 and 109.

PhtD

PhtD is the Pneumococcal histidine triad protein D. For reference purposes, the amino acid sequence of full length PhtD precursor is SEQ ID NO: 57 herein. In the R6 genome PhtD is spr0907 [89].

Preferred PhtD polypeptides 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: 57; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 57, 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). These PhtD proteins include variants of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 57 while retaining at least one epitope of SEQ ID NO: 57. Other fragments omit one or more protein domains.

The use of PhtD for immunisation is reported in references 107, 108 and 110.

PhtE

PhtE is the Pneumococcal histidine triad protein E. For reference purposes, the amino acid sequence of full length PhtE precursor is SEQ ID NO: 58 herein. In the R6 genome PhtE is spr0908 [89].

Preferred PhtE polypeptides 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: 58; and/or (b) comprising a fragment of at least 'n' consecutive amino acids of SEQ ID NO: 58, 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). These PhtE proteins include variants of SEQ ID NO: 58. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 58. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 58 while retaining at least one epitope of SEQ ID NO: 58. Other fragments omit one or more protein domains.

The use of PhtE for immunisation is reported in references 107 and 108.

Hybrid polypeptides

Where alternative meningococcal or pneumococcal antigens are used with the invention, these may be present in the composition as individual separate polypeptides. Where more than one such antigen

is used, however, they do not have to be present as separate polypeptides. Instead, at least two (*e.g.* 2, 3, 4, 5, or more) antigens can be expressed as a single polypeptide chain (a 'hybrid' polypeptide), as disclosed for meningococcal antigens in reference 111. Hybrid polypeptides offer two main advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The hybrid polypeptide may comprise two or more meningococcal or pneumococcal polypeptide sequences as disclosed above. Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten antigens are useful. In particular, hybrids consisting of amino acid sequences from two, three, four, or five antigens are preferred, such as two or three antigens.

Hybrid polypeptides can be represented by the formula NH_2-A-{-X-L-}$_n$-B-COOH, wherein: X is an amino acid sequence of an alternative meningococcal or pneumococcal antigen, as described 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. Other suitable linkers, particularly for use as the final L_n are a Leu-Glu dipeptide or SEQ ID NO: 59.

-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.

A particularly useful combination of meningococcal polypeptide antigens is disclosed in references 111 and 112, and a composition of the invention may thus include 1, 2, 3, 4 or 5 of: (1) a 'NadA' protein; (2) a '936' protein; (3) a '953' protein; (4) a '287' protein, and (5) a fHBP protein. For instance, a composition may include: (i) a first polypeptide having amino acid sequence SEQ ID NO: 4; (ii) a second polypeptide having amino acid sequence SEQ ID NO: 5; and (iii) a third polypeptide having amino acid sequence SEQ ID NO: 6.

Adjuvant

Compositions of the invention may include an immunological adjuvant. Thus, for example, they may include an aluminium salt adjuvant or an oil-in-water emulsion (*e.g.* a squalene-in-water emulsion). Other adjuvants may also be used.

Suitable aluminium salts include hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), (*e.g.* see chapters 8 & 9 of ref. 113), or mixtures thereof. The salts can take any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), with adsorption of antigen to the salt being typical. 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 0.3 and 1mg/ml. A maximum of 0.85mg/dose is preferred. A preferred aluminium salt adjuvant for use with CS14 and meningococcus antigens is an aluminium phosphate.

Various oil-in-water emulsion adjuvants are known, and they typically include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5μm in diameter, and may even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220nm are preferred as they can be subjected to filter sterilization.

The invention can be used with oils such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils include nuts, seeds and grains. Peanut oil, soybean oil, coconut oil, and

olive oil, the most commonly available, exemplify the nut oils. Jojoba oil can be used *e.g.* obtained from the jojoba bean. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil and the like. In the grain group, corn oil is the most readily available, but the oil of other cereal grains such as wheat, oats, rye, rice, teff, triticale and the like may also be used. 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, while not occurring naturally in seed oils, may be prepared by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils. Fats and oils from mammalian milk are metabolizable and may therefore be used in the practice of this invention. The procedures for separation, purification, saponification and other means necessary for obtaining pure oils from animal sources are well known in the art. Most fish contain metabolizable oils which may be readily recovered. For example, cod liver oil, shark liver oils, and whale oil such as spermaceti exemplify several of the fish oils which may be used herein. A number of branched chain oils are synthesized biochemically in 5-carbon isoprene units and are generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoids known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, which is particularly preferred herein. Squalane, the saturated analog to squalene, is also a preferred oil. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Other preferred oils are the tocopherols. Mixtures of oils can be used.

Where a composition includes a tocopherol, any of the α, β, γ, δ, ε or ξ tocopherols can be used, but α-tocopherols are preferred. The tocopherol can take several forms *e.g.* different salts and/or isomers. Salts include organic salts, such as succinate, acetate, nicotinate, *etc.* D-α-tocopherol and DL-α-tocopherol can both be used. A preferred α-tocopherol is DL-α-tocopherol, and the preferred salt of this tocopherol is the succinate.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Preferred surfactants of the invention have a HLB of at least 10, preferably at least 15, and more preferably at least 16. The invention can be used with surfactants including, but not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. Preferred surfactants for including in the emulsion are Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100.

Mixtures of surfactants can be used *e.g.* Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another useful combination comprises laureth-9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1 %; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1 %, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20 %, preferably 0.1 to 10 % and in particular 0.1 to 1 % or about 0.5%.

Specific oil-in-water emulsion adjuvants useful with the invention include, but are not limited to:

- A sub-micron emulsion of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. This adjuvant is known as 'MF59' [114-116], as described in more detail in Chapter 10 of ref. 113 and chapter 12 of ref. 117. The MF59 emulsion may include citrate ions *e.g.* 10mM sodium citrate buffer.

- An emulsion of squalene, a tocopherol, and polysorbate 80 (Tween 80). The emulsion may include phosphate buffered saline. It may also include Span 85 (*e.g.* at 1%) and/or lecithin. These emulsions may have from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% Tween 80, and the weight ratio of squalene:tocopherol is preferably ≤ 1 as this provides a more stable emulsion. Squalene and Tween 80 may be present volume ratio of about 5:2 or at a weight ratio of about 11:5. One such emulsion can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90ml of this solution with a mixture of (5g of DL-α-tocopherol and 5ml squalene), then microfluidising the mixture. The resulting emulsion may have submicron oil droplets *e.g.* with an average diameter of between 100 and 250nm, preferably about 180nm. The emulsion may also include a 3-de-O-acylated monophosphoryl lipid A (3d-MPL). Another useful emulsion of this type may comprise, per human dose, 0.5-10 mg squalene, 0.5-11 mg tocopherol, and 0.1-4 mg polysorbate 80 [118].

- An emulsion of squalene, a tocopherol, and a Triton detergent (*e.g.* Triton X-100). The emulsion may also include a 3d-MPL. The emulsion may contain a phosphate buffer.

- An emulsion comprising a polysorbate (*e.g.* polysorbate 80), a Triton detergent (*e.g.* Triton X-100) and a tocopherol (*e.g.* an α-tocopherol succinate). The emulsion may include these three components at a mass ratio of about 75:11:10 (*e.g.* 750μg/ml polysorbate 80, 110μg/ml Triton X-100 and 100μg/ml α-tocopherol succinate), and these concentrations should include any contribution of these components from antigens. The emulsion may also include squalene. The emulsion may also include a 3d-MPL. The aqueous phase may contain a phosphate buffer.

- An emulsion of squalane, polysorbate 80 and poloxamer 401 ("Pluronic™ L121"). The emulsion can be formulated in phosphate buffered saline, pH 7.4. This emulsion is a useful delivery vehicle for muramyl dipeptides, and has been used with threonyl-MDP in the "SAF-1" adjuvant [119] (0.05-1% Thr-MDP, 5% squalane, 2.5% Pluronic L121 and 0.2% polysorbate 80). It can also be used without the Thr-MDP, as in the "AF" adjuvant [120] (5% squalane, 1.25% Pluronic L121 and 0.2% polysorbate 80). Microfluidisation is preferred.

- An emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (*e.g.* polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (*e.g.* a sorbitan ester or mannide ester, such as sorbitan monoleate or 'Span 80'). The emulsion is preferably thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm [121]. The emulsion may also include one or more of: alditol; a cryoprotective agent (*e.g.* a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. Such emulsions may be lyophilized.

- An emulsion having from 0.5-50% of an oil, 0.1-10% of a phospholipid, and 0.05-5% of a non-ionic surfactant. As described in reference 122, preferred phospholipid components are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin and cardiolipin. Sub-micron droplet sizes are advantageous.

- A sub-micron oil-in-water emulsion of a non-metabolisable oil (such as light mineral oil) and at least one surfactant (such as lecithin, Tween 80 or Span 80). Additives may be included, such as QuilA saponin, cholesterol, a saponin-lipophile conjugate (such as GPI-0100, described in reference 123, produced by addition of aliphatic amine to desacylsaponin via the carboxyl group of glucuronic acid), dimethyidioctadecylammonium bromide and/or N,N-dioctadecyl-N,N-bis (2-hydroxyethyl)propanediamine.

- An emulsion comprising a mineral oil, a non-ionic lipophilic ethoxylated fatty alcohol, and a non-ionic hydrophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [124].

- An emulsion comprising a mineral oil, a non-ionic hydrophilic ethoxylated fatty alcohol, and a non-ionic lipophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [124].

- An emulsion in which a saponin (*e.g.* QuilA or QS21) and a sterol (*e.g.* a cholesterol) are associated as helical micelles [125].

Oil-in-water emulsions can be used as adjuvants on their own, or as carriers for further immunostimulatory compounds *e.g.* immunostimulatory oligonucleotides, 3d-MPL, *etc.*

Pharmaceutical compositions

The invention is concerned with pharmaceutical compositions for administration to a patient. These will typically include a pharmaceutically acceptable carrier. A thorough discussion of pharmaceutically acceptable carriers is available in reference 126.

Effective dosage volumes can be routinely established, but a typical human dose of the composition has a volume of about 0.5ml *e.g.* for intramuscular injection. This is the dosage volume for the PREVNAR™ product, the RIVM OMV-based vaccine and MeNZB™. These dosage volumes are typical for intramuscular injection, but similar doses may be used for other delivery routes *e.g.* an intranasal OMV-based vaccine for atomisation may have a volume of about 100µl or about 130µl per spray, with four sprays administered to give a total dose of about 0.5ml.

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). The pH of the RIVM OMV-based vaccine is 7.4 [127], and a pH <7.5 is preferred for compositions of the invention. The RIVM OMV-based vaccine maintains pH by using a 10mM Tris/HCl buffer, and stable pH in compositions of the invention may be maintained by the use of a buffer *e.g.* a Tris buffer, a citrate buffer, phosphate buffer, or a histidine buffer. Thus compositions of the invention will generally include a 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 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. A dose of about 0.9 mg protein per ml is typical for OMV-based intranasal vaccines.

Meningococci and pneumococci 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 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 Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

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.

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 both meningococcus and pneumococcus (for at least the meningococcal immunotype(s) and pneumococcal serotype(s), including serotype 14, represented in the composition) 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: (i) a meningococcal LOS and a CS14, wherein the LOS and/or the CS14 do(es) not include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide; (ii) a meningococcal LOS and a CS14, but no adjuvant, wherein the LOS and the CS14 both include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide; (iii) a meningococcal LOS and a pneumococcal polypeptide antigen, wherein the LOS includes a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide and the pneumococcal polypeptide can elicit an immune response that is effective against serotype 14 pneumococcus; or (iv) a meningococcal polypeptide and a CS14 wherein the CS14 includes a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide and the meningococcal polypeptide can elicit an immune response that is effective against serogroup B meningococcus, in the manufacture of a medicament for raising an immune response in a mammal.

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

One way of checking efficacy of therapeutic treatment involves monitoring meningococcal and/or pneumococcal 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 [128]) 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.

In some embodiments of the invention, the pneumococcal and meningococcal antigens may be co-administered but separately *i.e.* the two antigens may be for simultaneous, separate or sequential administration. Typically, however, the two antigens will be admixed for simultaneous combined administration.

Further antigens

As well as containing meningococcal and pneumococcal antigens as discussed above, compositions may include antigen(s) from further pathogen(s). For example, the composition may comprise one or more of the following further antigens:

- 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 antigens.

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.

In addition, a composition may include further meningococcal or pneumococcal antigens.

In addition to a CS14 antigen, for instance, a composition can include capsular saccharides from one or more other pneumococcal serotype(s). Thus, in addition to serotype 14, a composition may include a capsular saccharide from one or more of the following pneumococcal serotypes: 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and/or 33F. A composition may include multiple serotypes *e.g.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or more serotypes in total. 7-valent, 9-valent, 10-valent, 11-valent and 13-valent conjugate combinations are already known in the art, as is a 23-valent unconjugated combination. For example, a 10-valent combination may include saccharide from 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.* 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.* all separately conjugated to CRM197, prepared as disclosed in references 129, 130 and 131. 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.

Where a composition includes more than one pneumococcal conjugate, each conjugate may use the same carrier protein or a different carrier protein. Reference 132 describes potential advantages when using different carrier proteins in multivalent pneumococcal conjugate vaccines.

Where a composition includes saccharide antigens from more than one serotype, these are preferably prepared separately, conjugated separately, and then combined.

In addition to a meningococcal LOS or polypeptide antigen, a composition may include a meningococcal capsular saccharide, which will usually be conjugated to a carrier protein. 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.* Components including saccharides from all four 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 [133], 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 [134,135].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 [136-138]. 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 [139]. 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 [139]. 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.* [140], 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 and linkage chemistries are discussed above. Useful carriers include diphtheria toxoid, tetanus toxoid and CRM197.

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 141, 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.

As described in reference 142, 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.

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 143-149, *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 [150,151] or similar methods), or they can be predicted (*e.g.* using the Jameson-Wolf antigenic index [152], matrix-based approaches [153], MAPITOPE [154], TEPITOPE [155,156], neural networks [157], OptiMer & EpiMer [158,159], ADEPT [160], Tsites [161], hydrophilicity [162], antigenic index [163] or the methods disclosed in references 164-168, *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. 169. 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. 170.

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.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the core saccharides for a L3 immunotype.

Figure 2 shows the repeating structure of CS14 from reference 55.

Figure 3 shows anti-LNnT IgG titres in animal groups. Similarly, Figure 4 shows anti-CP14 IgG titres. Both figures show GMT values measured in RLU/ml.

MODES FOR CARRYING OUT THE INVENTION

Mice were immunised with (i) the PREVNAR™ vaccine at 0.4μg dose; (ii) outer membrane vesicles prepared from wild-type strain MC58 of serogroup B meningococcus at 10μg dose, combined with PREVNAR™; or (iii) outer membrane vesicles prepared from strain MC58 of serogroup B meningococcus having a *ΔlgtB* knockout at 10μg dose, combined with PREVNAR™. All compositions were formulated by adding aluminium hydroxide adjuvant.

Sera were analysed using a Luminex assay for antibodies against *inter alia* the pneumococcal serotype 14 capsular saccharide and the LNnT epitope.

Figure 3 shows anti-LNnT responses for the three groups on day 34 after immunization on days 1 and 21. The PREVNAR™ vaccine alone elicited an anti-LNnT response about 2x a blank control (0.2 *vs.* 0.12). Addition of the LOS-containing OMVs increased the response (0.7), but in $LgtB^{-ve}$ OMVs the increase was lower (0.5). The increase in group (iii) *vs.* group (i) could be due to a possible adjuvant effect of the OMVs (*cf.* reference 51).

Figure 4 shows anti-CS14 responses on day 34. Both of the OMV preparations increased the anti-CS14 responses; advantageously, a greater increase was seen with the $LgtB^{-ve}$ OMVs.

Although these data are incomplete and not fully conclusive, and the anti-LNnT assay gave only low signal, they show that CS14 and $LgtB^{-ve}$ OMVs can be combined to give better results than when combining CS14 and wild-type OMVs.

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.

REFERENCES

[1] van den Dobbelsteen *et al.* (2007) *Vaccine* 25:2491-6.
[2] Ram *et al.* (2003) *J Biol Chem* 278:50853-62.
[3] WO2004/014417.
[4] WO96/10086.
[5] US-5705161.
[6] WO2005/107798.
[7] US 6,531,131.
[8] WO98/53851
[9] US-6531131
[10] WO00/26384.
[11] US-6645503
[12] WO03/070282.
[13] WO94/08021
[14] *Research Disclosure*, 453077 (Jan 2002)
[15] EP-A-0372501.
[16] EP-A-0378881.
[17] EP-A-0427347.
[18] WO93/17712
[19] WO94/03208.
[20] WO98/58668.
[21] EP-A-0471177.
[22] WO91/01146
[23] Falugi *et al.* (2001) *Eur J Immunol* 31:3816-3824.
[24] Baraldo *et al.* (2004) *Infect Immun* 72(8):4884-7.
[25] EP-A-0594610.
[26] Ruan *et al.* (1990) *J Immunol* 145:3379-3384.
[27] WO00/56360.
[28] Kuo *et al.* (1995) *Infect Immun* 63:2706-13.
[29] Michon *et al.* (1998) *Vaccine.* 16:1732-41.
[30] WO02/091998.
[31] WO01/72337
[32] WO00/61761.
[33] WO00/33882
[34] WO02/09643.
[35] Katial *et al.* (2002) *Infect. Immun.* 70:702-707.
[36] US patent 6,180,111.
[37] WO01/34642.
[38] WO2004/019977.
[39] European patent 0011243.
[40] Fredriksen *et al.* (1991) *NIPH Ann.* 14(2):67-80.
[41] WO01/91788.
[42] WO2005/004908.
[43] Maiden *et al.* (1998) *PNAS USA* 95:3140-3145.
[44] WO98/56901.
[45] WO2006/081259.

[46] Claassen *et al.* (1996) 14(10):1001-8.
[47] WO2004/015099.
[48] WO01/52885.
[49] WO00/25811.
[50] WO01/09350.
[51] WO02/09746.
[52] WO02/062378.
[53] WO2007/144316.
[54] WO2007/144317.
[55] Wessels & Kasper (1989) *J Exp Med* 169:2121-31.
[56] WO2007/116028.
[57] *Mol. Immunol.*, 1985, **22**, 907-919
[58] EP-A-0208375
[59] Bethell G.S. *et al.*, *J. Biol. Chem.*, 1979, **254**, 2572-4
[60] Hearn M.T.W., *J. Chromatogr.*, 1981, **218**, 509-18
[61] WO00/10599
[62] Gever et al., Med. Microbiol. Immunol, 165 : 171-288 (1979).
[63] US patent 4,057,685.
[64] US patents 4,673,574; 4,761,283; 4,808,700.
[65] US patent 4,459,286.
[66] US patent 5,204,098
[67] US patent 4,965,338
[68] US patent 4,663,160.
[69] WO2007/000343.
[70] Jodar *et al.* (2003) *Vaccine* 21:3265-72.
[71] Masignani *et al.* (2003) *J Exp Med* 197:789-799.
[72] Welsch *et al.* (2004) *J Immunol* 172:5605-15.
[73] Hou *et al.* (2005) *J Infect Dis* 192(4):580-90.
[74] WO03/063766.
[75] Fletcher *et al.* (2004) *Infect Immun* 72:2088-2100.
[76] Zhu *et al.* (2005) *Infect Immun* 73(10):6838-45.
[77] Cantini *et al.* (2006) *J. Biol. Chem.* 281:7220-7227
[78] WO2004/048404
[79] Tettelin *et al.* (2000) *Science* 287:1809-1815.
[80] WO00/66741.
[81] WO99/57280
[82] Martin *et al.* (1997) *J Exp Med* 185(7):1173-83.
[83] WO96/29412.
[84] US-5,698,438.
[85] Perkins-Balding *et al.* (2003) *Microbiology* 149:3423-35.
[86] WO01/55182.
[87] WO01/38350.
[88] WO00/23595.
[89] Hoskins *et al.* (2001) *J.Bacteriol.* 183:5709-5717.
[90] WO2004/092209.
[91] Kirkham *et al.* (2006) *Infect Immun.* 74(1):586-93.

[92] WO2005/108580.
[93] Berry *et al.* (1999) *Infect Immun* 67(2):981-5.
[94] US-6716432.
[95] WO90/06951.
[96] WO99/03884.
[97] Baba *et al.* (2002) *Infect Immun* 70: 107-113.
[98] US-7217791
[99] WO2008/061953.
[100] Briles *et al.* (2000) *J Infect Dis* 182:1694-1701.
[101] Talkington *et al.* (1996) *Microb Pathog.* 21(1):17-22.
[102] WO99/53940.
[103] WO02/22168.
[104] WO02/22167.
[105] WO02/08426.
[106] WO2003/104272.
[107] WO00/37105.
[108] Adamou *et al.* (2001) *Infect Immun.* 69(2):949-58.
[109] Ogunniyi *et al.* (2007) *Infect Immun.* 75(1):350-7.
[110] WO98/18930.
[111] Giuliani *et al.* (2006) *PNAS USA* 103:10834-9.
[112] WO2004/032958.
[113] *Vaccine Design...* (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.
[114] WO90/14837.
[115] Podda & Del Giudice (2003) *Expert Rev Vaccines* 2:197-203.
[116] Podda (2001) *Vaccine* 19: 2673-2680.
[117] *Vaccine Adjuvants: Preparation Methods and Research Protocols* (Volume 42 of *Methods in Molecular Medicine* series). ISBN: 1-59259-083-7. Ed. O'Hagan.
[118] WO2008/043774.
[119] Allison & Byars (1992) *Res Immunol* 143:519-25.
[120] Hariharan *et al.* (1995) *Cancer Res* 55:3486-9.
[121] US-2007/0014805.
[122] WO95/11700.
[123] US patent 6,080,725.
[124] WO2006/113373.
[125] WO2005/097181.
[126] Gennaro (2000) *Remington: The Science and Practice of Pharmacy.* 20th edition, ISBN: 0683306472.
[127] RIVM report 000012 003.
[128] WO01/30390.
[129] US-2007/0231340.
[130] US-2007/0184072.
[131] US-2006/0228380.
[132] WO2007/071707
[133] WO03/080678.
[134] Glode *et al.* (1979) *J Infect Dis* 139:52-56
[135] WO94/05325; US patent 5,425,946.
[136] Arakere & Frasch (1991) *Infect. Immun.* 59:4349–4356.
[137] Michon *et al.* (2000) *Dev. Biol.* 103:151–160.

[138] Rubinstein & Stein (1998) *J. Immunol.* 141:4357–4362.

[139] WO2005/033148

[140] WO2007/000314.

[141] WO2007/000341.

[142] WO2007/000342.

[143] *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.)

[144] *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds, 1986, Blackwell Scientific Publications)

[145] Sambrook *et al.* (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition (Cold Spring Harbor Laboratory Press).

[146] *Handbook of Surface and Colloidal Chemistry* (Birdi, K.S. ed., CRC Press, 1997)

[147] Ausubel *et al.* (eds) (2002) *Short protocols in molecular biology*, 5th edition (Current Protocols).

[148] *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream *et al.*, eds., 1998, Academic Press)

[149] *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag)

[150] Geysen *et al.* (1984) *PNAS USA* 81:3998-4002.

[151] Carter (1994) *Methods Mol Biol* 36:207-23.

[152] Jameson, BA *et al.* 1988, *CABIOS* 4(1):181-186.

[153] Raddrizzani & Hammer (2000) *Brief Bioinform* 1(2):179-89.

[154] Bublil *et al.* (2007) *Proteins* 68(1):294-304.

[155] De Lalla *et al.* (1999) *J. Immunol.* 163:1725-29.

[156] Kwok *et al.* (2001) *Trends Immunol* 22:583-88.

[157] Brusic *et al.* (1998) *Bioinformatics* 14(2):121-30

[158] Meister *et al.* (1995) *Vaccine* 13(6):581-91.

[159] Roberts *et al.* (1996) *AIDS Res Hum Retroviruses* 12(7):593-610.

[160] Maksyutov & Zagrebelnaya (1993) *Comput Appl Biosci* 9(3):291-7.

[161] Feller & de la Cruz (1991) *Nature* 349(6311):720-1.

[162] Hopp (1993) *Peptide Research* 6:183-190.

[163] Welling *et al.* (1985) *FEBS Lett.* 188:215-218.

[164] Davenport *et al.* (1995) *Immunogenetics* 42:392-297.

[165] Tsurui & Takahashi (2007) *J Pharmacol Sci.* 105(4):299-316.

[166] Tong *et al.* (2007) *Brief Bioinform.* 8(2):96-108.

[167] Schirle *et al.* (2001) *J Immunol Methods.* 257(1-2):1-16.

[168] Chen *et al.* (2007) *Amino Acids* 33(3):423-8.

[169] *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30

[170] Smith & Waterman (1981) *Adv. Appl. Math.* 2: 482-489.

CLAIMS

1. An immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal serotype 14 capsular saccharide (CS14), wherein the LOS and/or the CS14 do(es) not include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide.

2. The composition of claim 1, in which the tetrasaccharide is present in CS14.

3. The composition of claim 1 or claim 2, including an adjuvant.

4. The composition of any preceding claim, wherein the LOS is prepared from a meningococcal strain lacking LgtB enzyme activity.

5. The composition of any preceding claim, wherein the LOS is prepared from a meningococcal strain lacking GalE enzyme activity.

6. The composition of any preceding claim, wherein the LOS is prepared from a meningococcal strain lacking LgtA and/or LgtE enzyme activity.

7. The composition of any preceding claim, wherein the LOS lacks the terminal galactose of the Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide.

8. The composition of any preceding claim, wherein the LOS is present within meningococcal outer membrane vesicles.

9. The composition of claim 8, wherein the LOS is conjugated to proteins in the vesicles.

10. The composition of claim 8 or claim 9, wherein the vesicles are prepared from a meningococcus that over-expresses TbpA.

11. The composition of any one of claims 1 to 6, wherein the LOS is conjugated to a carrier protein.

12. The composition of claim 11, wherein conjugation may be via a lipid A portion in the LOS or by a KDO residue.

13. An immunogenic composition comprising one or more meningococcal polypeptide(s) and a pneumococcal serotype 14 capsular saccharide, wherein (i) the pneumococcal serotype 14 capsular saccharide includes a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide, (ii) the meningococcal polypeptide can elicit an immune response that is effective against serogroup B meningococcus, and (iii) the composition does not include a meningococcal lipooligosaccharide.

14. The composition of claim 13, wherein the meningococcal polypeptide(s) comprise a fHBP.

15. The composition of claim 14, wherein the fHBP comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 1 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 1.

16. The composition of claim 14, wherein the fHBP comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 2 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 2.

17. The composition of claim 14, wherein the fHBP comprises an amino acid sequence having at least 85% sequence identity to SEQ ID NO: 3 and/or comprising an amino acid sequence consisting of a fragment of at least 7 contiguous amino acids from SEQ ID NO: 3.

18. The composition of claim 14, 15, 16 or 17, wherein the fHBP is lipidated.

19. The composition of claim 14, 15, 16, 17 or 18, wherein the fHBP elicits antibodies which can bind to a meningococcal polypeptide consisting of amino acid sequence SEQ ID NO: 1, 2 or 3.

20. An unadjuvanted immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal serotype 14 capsular saccharide (CS14), wherein the LOS and the CS14 both include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide.

21. An immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal serotype 14 capsular saccharide (CS14), wherein (i) the LOS and the CS14 both include a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide, (ii) the concentration of LOS is less than 5μg/ml, and (iii) the concentration of CS14 is less than 5μg/ml.

22. The composition of any preceding claim, wherein the CS14 is conjugated to a carrier protein.

23. The composition of claim 22, wherein the carrier protein is CRM197, tetanus toxoid, diphtheria toxoid or *H.influenzae* protein D.

24. An immunogenic composition comprising a meningococcal lipooligosaccharide (LOS) and a pneumococcal polypeptide antigen, wherein (i) the LOS includes a Galβ1-4GlcNAcβ1-3Galβ1-4Glc tetrasaccharide, (ii) the pneumococcal polypeptide can elicit an immune response that is effective against serotype 14 pneumococcus, and (iii) the composition does not include a pneumococcal serotype 14 capsular saccharide.

25. The composition of any one of claims 1-19, 21-24 when dependent on claims 1-19, including an aluminium salt adjuvant.

26. The composition of claim 25, wherein the aluminium salt is an aluminium phosphate.

27. A method for raising an immune response in a mammal, comprising administering a composition of any preceding claim to the mammal.

FIGURE 1

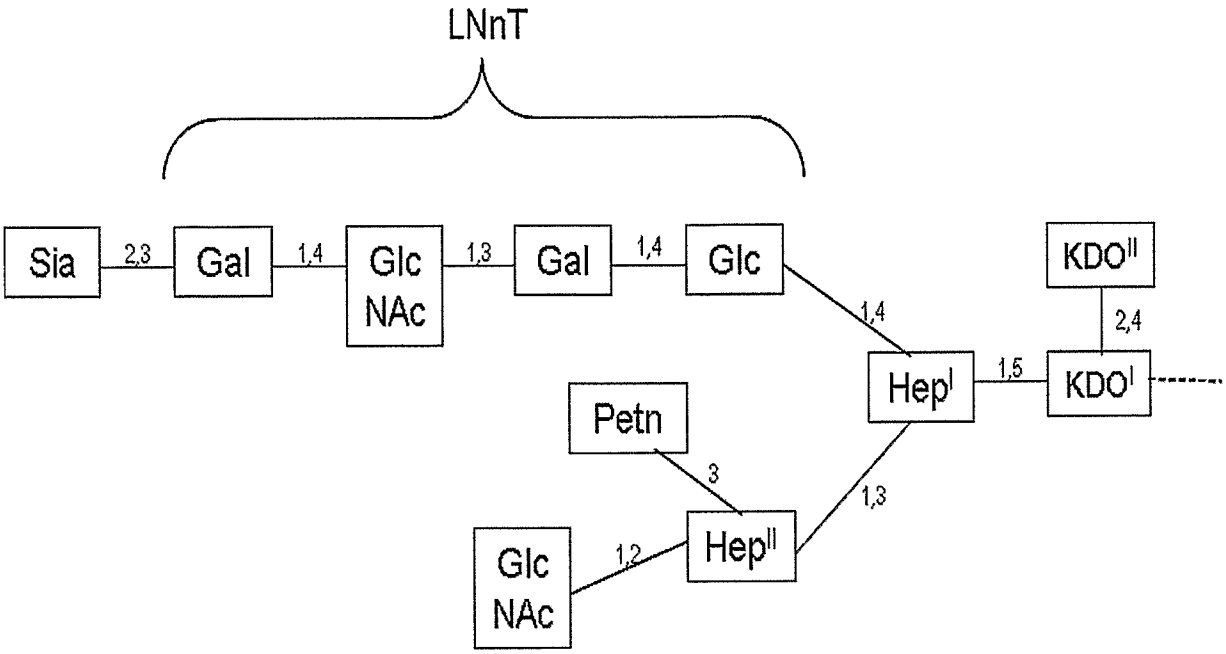


FIGURE 2

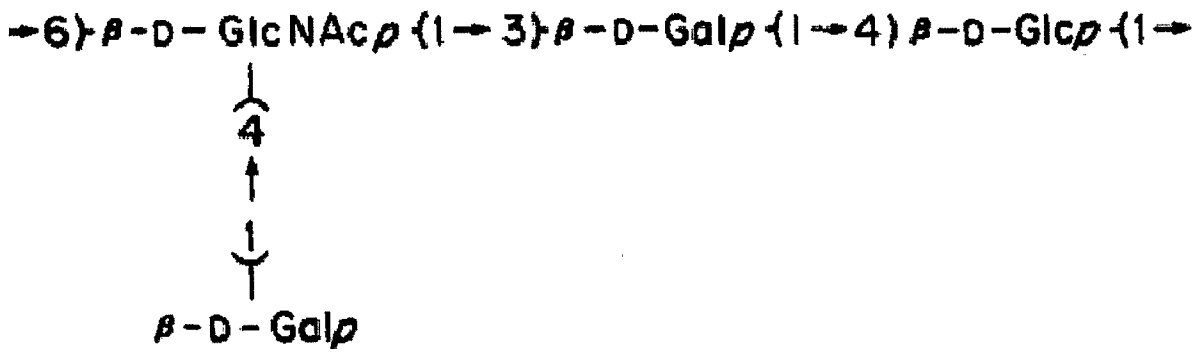


FIGURE 3

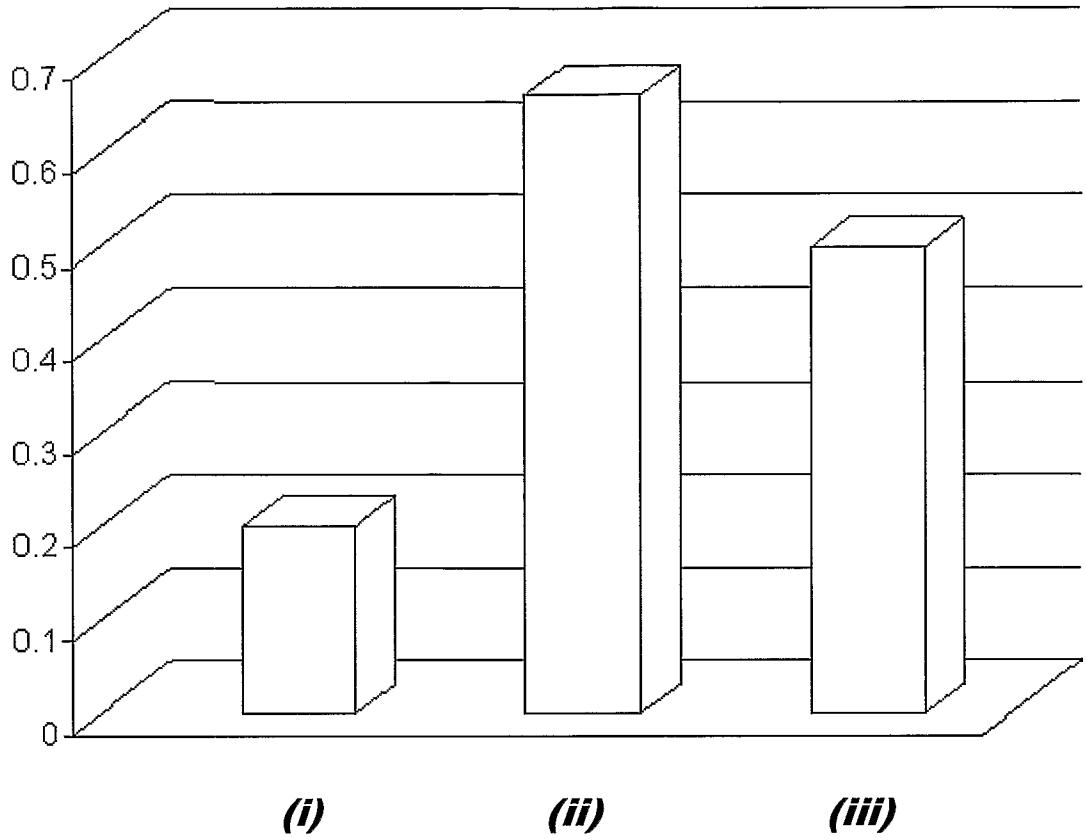


FIGURE 4