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COMANDUCCI MAURIZIO ET AL: "NadA, a novel vaccine candidate of Neisseria meningitidis", JOURNAL OF EXPERIMENTAL MEDICINE, vol. 195, no. 11, 3 June 2002 (2002-06-03) , pages 1445-1454, XP002272871, ISSN: 0022-1007
GRIFANTINI RENATA ET AL: "Previously unrecognized vaccine candidates against group B meningococcus identified by DNA microarrays", NATURE BIOTECHNOLOGY, vol. 20, no. 9, September 2002 (2002-09), pages 914-921, XP002272872, ISSN: 1087-0156
MAGAGNOLI C ET AL: "Structural organization of NadADelta351-405, a recombinant MenB vaccine component, by its physico-chemical characterization at drug substance level", VACCINE, ELSEVIER LTD, GB, vol. 27, no. 15, 26 March 2009 (2009-03-26), pages 2156-2170, XP025969092, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2009.01.099 [retrieved on 2009-01-31]

DESCRIPTION

TECHNICAL FIELD

[0001] This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens from *Neisseria meningitidis* (meningococcus) and their use in immunisation.

BACKGROUND ART

[0002] *N.meningitidis* is a non-motile, Gram-negative human pathogen that colonises the pharynx and causes meningitis (and, occasionally, septicaemia in the absence of meningitis). It causes both endemic and epidemic disease. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N.meningitidis* is the major cause of bacterial meningitis in the USA.

[0003] Based on the organism's capsular polysaccharide, various serogroups of *N.meningitidis* have been identified. Serogroup A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the USA and developed countries. After serogroup, classification includes serotype, serosubtype and then immunotype, and the standard nomenclature lists serogroup, serotype, serosubtype, and immunotype, each separated by a colon e.g. B:4:P1.15:L3,7,9. Within serogroup B, some lineages cause disease often (hyperinvasive), some lineages cause more severe forms of disease than others (hypervirulent), and others rarely cause disease at all. Seven hypervirulent lineages are recognised, namely subgroups I, III and IV-1, ET-5 complex, ET-37 complex, A4 cluster and lineage 3. These have been defined by multilocus enzyme electrophoresis (MLEE), but multilocus sequence typing (MLST) has also been used to classify meningococci [ref. 1].

[0004] A polysaccharide vaccine against serogroups A, C, W135 & Y has been known for many years [2, 3] but a vaccine against serogroup B has proved elusive. Vaccines based on outer-membrane vesicles have been tested [e.g. see ref. 4], but the protection afforded by these vaccines is typically restricted to the strain used to make the vaccine. There remains a need, therefore, for a broadly-effective serogroup B vaccine.

[0005] Genome sequences for meningococcal serogroups A [5] and B [6,7] have been reported, and the serogroup B sequence has been studied to identify vaccine antigens [e.g. refs. 8 to 13]. Candidate antigens have been manipulated to improve heterologous expression [refs. 14 to 16].

[0006] It is an object of the invention to provide further and improved compositions for providing immunity against meningococcal disease and/or infection, and in particular for providing broad immunity against serogroup B meningococcus.

DISCLOSURE OF THE INVENTION

[0007] Vaccines against pathogens such as hepatitis B virus, diphtheria and tetanus typically contain a single protein antigen (e.g. the HBV surface antigen, or a tetanus toxoid). In contrast, acellular whooping cough vaccines typically contain at least three *B.pertussis* proteins and the Prevenar™ pneumococcal vaccine contains seven separate conjugated saccharide antigens. Other vaccines such as cellular pertussis vaccines, the measles vaccine, the inactivated polio vaccine (IPV) and meningococcal OMV vaccines are by their very nature complex mixtures of a large number of antigens.

[0008] Whether protection against can be elicited by a single antigen, a small number of defined antigens, or a complex mixture of undefined antigens, therefore depends on the pathogen in question. The invention is based on the discovery that a small number of defined antigens is able to provide broad protection against meningococcal infection, and the invention provides a composition which, after administration to a subject, is able to induce an antibody response in that subject, wherein the antibody response is bactericidal against two or more (e.g. 2 or 3) of hypervirulent lineages A4, ET-5 and lineage 3 of *N.meningitidis* serogroup B.

[0009] Rather than consisting of a single antigen, it is preferred that the composition of the invention comprises a mixture of 10 or fewer (e.g. 9, 8, 7, 6, 5, 4, 3, 2) purified antigens, and it is particularly preferred that the composition should not include

complex or undefined mixtures of antigens e.g. it is preferred not to include outer membrane vesicles in the composition.

[0010] For serogroup B meningococcus, a mixture of five defined protein antigens has been found to elicit a good protective immune response. The invention thus provides a composition as defined in the claims comprising the following five meningococcal protein antigens: (1) a 'NadA' protein; (2) a '741' protein; (3) a '936' protein; (4) a '953' protein; and (5) a '287' protein. These antigens are referred to herein as the 'five basic antigens'.

NadA protein

[0011] 'NadA' (Neisserial adhesin A) from serogroup B of *N.meningitidis* is disclosed as protein '961' in reference 10 (SEQ IDs 2943 & 2944) and as 'NMB1994' in reference 6 (see also GenBank accession numbers: 11352904 & 7227256). A detailed description of the protein can be found in reference 17. There is no corresponding protein in serogroup A [5, 17].

[0012] When used according to the present invention, NadA is a polypeptide consisting of the amino acid sequence of SEQ ID NO:2.

[0013] NadA may take various forms including truncation or deletion variants, such as those disclosed in references 14 to 16. In particular, NadA without its C-terminal membrane anchor is preferred (e.g. deletion of residues 351-405 for strain 2996 [SEQ ID 1]), which is sometimes distinguished herein by the use of a 'C' superscript e.g. NadA^(C). Expression of NadA without its membrane anchor domain (e.g. SEQ ID 1) in *E.coli* results in secretion of the protein into the culture supernatant with concomitant removal of its 23mer leader peptide (e.g. to leave a 327mer for strain 2996 [SEQ ID 2]). Polypeptides without their leader peptides are sometimes distinguished herein by the use of a 'NL' superscript e.g. NadA^(NL) or NadA^{(C)(NL)}.

[0014] Allelic forms of NadA are shown in Figure 9 of reference 18.

[0015] Where N-terminus residues are deleted, it is preferred that the deletion should not remove the ability of NadA to adhere to human epithelial cells. A preferred fragment of SEQ ID 1 is SEQ ID 2.

[0016] Secreted NadA can conveniently be prepared in highly pure form from culture supernatant by a process comprising the steps of: concentration and diafiltration against a buffer by ultrafiltration; anionic column chromatography; hydrophobic column chromatography; hydroxylapatite ceramic column chromatography; diafiltration against a buffer; and filter sterilisation. Further details of the process are given in the examples.

[0017] NadA is preferably used in an oligomeric form (e.g. in trimeric form).

741 protein

[0018] '741' protein from serogroup B is disclosed in reference 10 (SEQ IDs 2535 & 2536) and as 'NMB1870' in reference 6 (see also GenBank accession number GI:7227128). The corresponding protein in serogroup A [5] has GenBank accession number 7379322. 741 is naturally a lipoprotein.

[0019] When used according to the present invention, 741 protein has ≥85% identity to SEQ ID NO: 3.

[0020] 741 protein may take various forms including truncation or deletion variants, such as those disclosed in references 14 to 16. In particular, the N-terminus of 741 may be deleted up to and including its poly-glycine sequence (i.e. deletion of residues 1 to 72 for strain MC58 [SEQ ID 3]), which is sometimes distinguished herein by the use of a 'ΔG' prefix. This deletion can enhance expression. The deletion also removes 741's lipidation site.

[0021] Preferred 741 sequences have 90% or more identity (e.g. 95%, 99% or more) to SEQ ID 3. This includes 741 variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.). Allelic forms of 741 can be found in SEQ IDs 1 to 22 of reference 16, and in SEQ IDs 1 to 23 of reference 19. SEQ IDs 1-299 of reference 20 give further 741 sequences.

[0022] Protein 741 is an extremely effective antigen for eliciting anti-meningococcal antibody responses, and it is expressed across all meningococcal serogroups. Phylogenetic analysis shows that the protein splits into two groups, and that one of these

splits again to give three variants in total [21], and while serum raised against a given variant is bactericidal within the same variant group, it is not active against strains which express one of the other two variants *i.e.* there is intra-variant cross-protection, but not inter-variant cross-protection. For maximum cross-strain efficacy, therefore, it is preferred that a composition should include more than one variant of protein 741. An exemplary sequence from each variant is given in SEQ ID 10, 11 and 12 herein, starting with a N-terminal cysteine residue to which a lipid will be covalently attached in the lipoprotein form of 741.

[0023] It is therefore preferred that the composition should include: (1) a first protein, comprising an amino acid sequence having at least *a*% sequence identity to SEQ ID 10; and at least one of: (2) a second protein, comprising an amino acid sequence having at least *b*% sequence identity to SEQ ID 11 and/or comprising an amino acid sequence consisting of a fragment of at least *y* contiguous amino acids from SEQ ID 11; and (3) a third protein, comprising an amino acid sequence having at least *c*% sequence identity to SEQ ID 12 and/or comprising an amino acid sequence consisting of a fragment of at least *z* contiguous amino acids from SEQ ID 12.

[0024] 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.

[0025] 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.

[0026] It is preferred that any given 741 amino acid sequence will not fall into more than one of categories (1), (2) and (3). Any given 741 sequence will thus fall into only one of categories (1), (2) and (3). It is thus preferred that: protein (1) has less than *i*% sequence identity to protein (2); protein (1) has less than *j*% sequence identity to protein (3); and protein (2) has less than *k*% sequence identity to protein (3). The value of *i* is 60 or more (*e.g.* 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, *etc.*) and is at most *a*. The value of *j* is 60 or more (*e.g.* 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, *etc.*) and is at most *b*. The value of *k* is 60 or more (*e.g.* 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, *etc.*) and is at most *c*. The values of *i*, *j* and *k* are not intrinsically related to each other.

936 protein

[0027] '936' protein from serogroup B is disclosed in reference 10 (SEQ IDs 2883 & 2884) and as 'NMB2091' in reference 6 (see also GenBank accession number GI:7227353). The corresponding gene in serogroup A [5] has GenBank accession number 7379093.

[0028] When used according to the present invention, 936 protein has ≥85% identity to SEQ ID NO: 4.

[0029] 936 protein may take various forms including truncation or deletion variants, such as those disclosed in references 14 to 16. In particular, the N-terminus leader peptide of 936 may be deleted (*i.e.* deletion of residues 1 to 23 for strain MC58 [SEQ ID 4]) to give 936^(NL).

[0030] Preferred 936 sequences have 90% or more identity (*e.g.* 95%, 99% or more) to SEQ ID 4. This includes variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants *etc.*).

953 protein

[0031] '953' protein from serogroup B is disclosed in reference 10 (SEQ IDs 2917 & 2918) and as 'NMB1030' in reference 6 (see also GenBank accession number GI:7226269). The corresponding protein in serogroup A [5] has GenBank accession number 7380108.

[0032] When used according to the present invention, 953 protein has ≥85% identity to SEQ ID NO: 5.

[0033] 953 protein may take various forms. Preferred forms of 953 are truncation or deletion variants, such as those disclosed in

references 14 to 16. In particular, the N-terminus leader peptide of 953 may be deleted (*i.e.* deletion of residues 1 to 19 for strain MC58 [SEQ ID 5]) to give 953^(NL).

[0034] Preferred 953 sequences have 90% or more identity (*e.g.* 95%, 99% or more) to SEQ ID 5. This includes 953 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 953 can be seen in Figure 19 of reference 12.

287 protein

[0035] '287' protein from serogroup B is disclosed in reference 10 (SEQ IDs 3103 & 3104), as 'NMB2132' in reference 6, and as 'GNA2132' in reference 13 (see also GenBank accession number GI:7227388). The corresponding protein in serogroup A [5] has GenBank accession number 7379057.

[0036] When used according to the present invention, 287 protein has $\geq 85\%$ identity to SEQ ID NO: 6.

[0037] 287 protein may take various forms including truncation or deletion variants, such as those disclosed in references 14 to 16. In particular, the N-terminus of 287 may be deleted up to and including its poly-glycine sequence (*i.e.* deletion of residues 1 to 24 for strain MC58 [SEQ ID 6]), which is sometimes distinguished herein by the use of a ' Δ G' prefix. This deletion can enhance expression.

[0038] Preferred 287 sequences have 90% or more identity (*e.g.* 95%, 99% or more) to SEQ ID 6. This includes 287 variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*). Allelic forms of 287 can be seen in Figures 5 and 15 of reference 12, and in example 13 and figure 21 of reference 10 (SEQ IDs 3179 to 3184).

Fusion proteins

[0039] The five antigens may be present in the composition as five separate proteins, but it is preferred that at least two of the antigens are expressed as a single polypeptide chain (a 'hybrid' protein [refs. 14 to 16]) *e.g.* such that the five antigens form fewer than five polypeptides. Hybrid proteins offer two principal advantages: first, a protein 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 separately-useful proteins.

[0040] A hybrid protein included in a composition of the invention may comprise two or more (*i.e.* 2, 3, 4 or 5) of the five basic antigens. Hybrids consisting of two of the five basic antigens are preferred.

[0041] Within the combination of five basic antigens, an antigen may be present in more than one hybrid protein and/or as a non-hybrid protein. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both, although it may be useful to include protein 741 both as a hybrid and a non-hybrid (preferably lipoprotein) antigen, particularly where more than one variant of 741 is used.

[0042] Two-antigen hybrids for use in the invention comprise: NadA & 741; NadA & 936; NadA & 953; NadA & 287; 741 & 936; 741 & 953; 741 & 287; 936 & 953; 936 & 287; 953 & 287. Preferred two-antigen hybrids comprise: 741 & 936; 953 & 287.

[0043] Hybrid proteins can be represented by the formula **NH₂-A-[-X-L-]_n-B-COOH**, wherein: X is an amino acid sequence of one of the five basic antigens; 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; and *n* is 2, 3, 4 or 5.

[0044] 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 X₁ will be retained, but the leader peptides of X₂ ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X₁ as moiety -A-.

[0045] 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 NH₂-X₁-L₁-X₂-L₂-COOH, NH₂-X₁-X₂-COOH, NH₂-X₁-L₁-X₂-COOH, NH₂-X₁-X₂-L₂-COOH, *etc.* Linker amino acid

sequence(s) -L- will typically be short (e.g. 20 or fewer amino acids *i.e.* 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 9), 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. If X_{n+1} is a ΔG protein and L_n is a glycine linker, this may be equivalent to X_{n+1} not being a ΔG protein and L_n being absent.

[0046] -A- is an optional N-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 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 X₁ 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.

[0047] -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), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

[0048] Most preferably, *n* is 2. Two preferred proteins of this type are: X₁ is a 936 and X₂ is a 741; X₁ is a 287 and X₂ is a 953.

[0049] Two particularly preferred hybrid proteins of the invention are as follows:

n	A	X ₁	L ₁	X ₂	L ₂	B	[SEQ ID]
2	MA	ΔG287	GSGGGG	953(NL)	-	-	7
2	M	936(NL)	GSGGGG	ΔG741	-	-	8

These two proteins may be used in combination with NadA (SEQ ID 2).

[0050] 936-ΔG741 hybrid can conveniently be prepared in highly pure form from expression in *E.coli* by a process comprising the steps of: homogenisation; centrifugation; cationic column chromatography; anionic column chromatography; hydrophobic column chromatography; diafiltration against a buffer; and filter sterilisation. Further details of the process are given in the examples.

[0051] Polypeptides can be prepared by various means (e.g. recombinant expression, purification from cell culture, chemical synthesis (at least in part), *etc.*) and in various forms (e.g. native, fusions, non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other *N.meningitidis* or host cell proteins).

Strains

[0052] Preferred proteins comprise an amino acid sequence found in *N.meningitidis* serogroup B. Within serogroup B, preferred strains are 2996, MC58, 95N477, and 394/98. Strain 394/98 is sometimes referred to herein as 'NZ', as it is a New Zealand strain.

[0053] Protein 287 is preferably from strain 2996 or, more preferably, from strain 394/98.

[0054] Protein 741 is preferably from serogroup B strains MC58, 2996, 394/98, or 95N477, or from serogroup C strain 90/18311. Strain MC58 is more preferred.

[0055] Proteins 936 and 953 are preferably from strain 2996. NadA is from strain 2996.

[0056] Strains may be indicated as a subscript e.g. 741_{MC58} is protein 741 from strain MC58. Unless otherwise stated, proteins mentioned herein (e.g. with no subscript) are from *N.meningitidis* strain 2996, which can be taken as a 'reference' strain. It will be appreciated, however, that the invention is not in general limited by strain. As mentioned above, general references to a protein (e.g. '287', '919' *etc.*) may be taken to include that protein from any strain. This will typically have sequence identity to 2996 of

90% or more (e.g. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

[0057] Where a composition includes a particular protein antigen (e.g. 741 or 287), the composition can include that antigen in more than one variant form e.g. the same protein, but from more than one strain. These proteins may be included as tandem or separate proteins.

[0058] Where hybrid proteins are used, the individual antigens within the hybrid (i.e. individual -X-moieties) may be from one or more strains. Where $n=2$, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where $n=3$, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2 \neq X_3$ (iii) $X_1 \neq X_2=X_3$ (iv) $X_1 \neq X_2 \neq X_3$ or (v) $X_1=X_3 \neq X_2$, etc.

Hypervirulent lineages and bactericidal antibody responses

[0059] In general, compositions of the invention are able to induce serum bactericidal antibody responses after being administered to a subject. These responses are conveniently measured in mice and are a standard indicator of vaccine efficacy [e.g. see end-note 14 of reference 13]. Serum bactericidal activity (SBA) measures bacterial killing mediated by complement, and can be assayed using human or baby rabbit complement. WHO standards require a vaccine to induce at least a 4-fold rise in SBA in more than 90% of recipients.

[0060] Rather than offering narrow protection, compositions of the invention can induce bactericidal antibody responses against more than one hypervirulent lineage of serogroup B. In particular, they can induce bactericidal responses against two or three of the following three hypervirulent lineages: (i) cluster A4; (ii) ET5 complex; and (iii) lineage 3. They may additionally induce bactericidal antibody responses against one or more of hypervirulent lineages subgroup I, subgroup III, subgroup IV-1 or ET-37 complex, and against other lineages e.g. hyperinvasive lineages.

[0061] This does not necessarily mean that the composition can induce bactericidal antibodies against each and every strain of serogroup B meningococcus within these hypervirulent lineages e.g. rather, for any given group of four or more strains of serogroup B meningococcus within a particular hypervirulent lineage, the antibodies induced by the composition are bactericidal against at least 50% (e.g. 60%, 70%, 80%, 90% or more) of the group. Preferred groups of strains will include strains isolated in at least four of the following countries: GB, AU, CA, NO, IT, US, NZ, NL, BR, and CU. The serum preferably has a bactericidal titre of at least 1024 (e.g. 2^{10} , 2^{11} , 2^{12} , 2^{13} , 2^{14} , 2^{15} , 2^{16} , 2^{17} , 2^{18} or higher, preferably at least 2^{14}) i.e. the serum is able to kill at least 50% of test bacteria of a particular strain when diluted 1/1024, as described in reference 13.

[0062] Preferred compositions can induce bactericidal responses against the following strains of serogroup B meningococcus: (i) from cluster A4, strain 961-5945 (B:2b:P1.21,16) and/or strain G2136 (B:-); (ii) from ET-5 complex, strain MC58 (B:15:P1.7,16b) and/or strain 44/76 (B:15:P1.7,16); (iii) from lineage 3, strain 394/98 (B:4:P1.4) and/or strain BZ198 (B:NT:-). More preferred compositions can induce bactericidal responses against strains 961-5945, 44/76 and 394/98.

[0063] Strains 961-5945 and G2136 are both *Neisseria* MLST reference strains [ids 638 & 1002 in ref. 22]. Strain MC58 is widely available (e.g. ATCC BAA-335) and was the strain sequenced in reference 6. Strain 44/76 has been widely used and characterised (e.g. ref. 23) and is one of the *Neisseria* MLST reference strains [id 237 in ref. 22; row 32 of Table 2 in ref. 1]. Strain 394/98 was originally isolated in New Zealand in 1998, and there have been several published studies using this strain (e.g. refs. 24 & 25). Strain BZ198 is another MLST reference strain [id 409 in ref. 22; row 41 of Table 2 in ref. 1].

[0064] The composition may additionally induce a bactericidal response against serogroup W135 strain LNP17592 (W135:2a:P1.5,2), from ET-37 complex. This is a Haji strain isolated in France in 2000.

Heterologous host

[0065] Whilst expression of the proteins may take place in *Neisseria*, preferably a heterologous host is utilised. The heterologous host may be prokaryotic (e.g. a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (e.g. *M.tuberculosis*), yeast, etc.

[0066] Thus the invention provides a composition as defined in the claims which, after administration to a subject, is able to

induce an antibody response in that subject, wherein the antibody response is bactericidal against two or more (e.g. 2 or 3) of hypervirulent lineages A4, ET-5 and lineage 3 of *N.meningitidis* serogroup B, and wherein the immunogens in the composition which give rise to the antibody response are obtained by recombinant expression in a non-Neisserial host. Thus the immunogens in the compositions of the invention are preferably recombinant immunogens. Compositions which do not include OMV preparations may thus be preferred.

Immunogenic compositions and medicaments

[0067] Compositions of the invention 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.

[0068] The pH of the composition is preferably between 6 and 8, preferably about 7. Stable pH may be maintained by the use of a buffer. Where a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [26]. The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

[0069] Compositions may be presented in vials, or they may be presented in ready-filled syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses. Injectable compositions will usually be liquid solutions or suspensions. Alternatively, they may be presented in solid form (e.g. freeze-dried) for solution or suspension in liquid vehicles prior to injection.

[0070] Compositions of the invention may be packaged in unit dose form or in multiple dose form. For multiple dose forms, vials are preferred to pre-filled syringes. Effective dosage volumes can be routinely established, but a typical human dose of the composition for injection has a volume of 0.5ml.

[0071] Where a composition of the invention is to be prepared extemporaneously prior to use (e.g. where a component is presented in lyophilised form) and is presented as a kit, the kit may comprise two vials, or it may comprise one ready-filled syringe and one vial, with the contents of the syringe being used to reactivate the contents of the vial prior to injection.

[0072] The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

[0073] The invention also provides the use of a composition of the invention in the manufacture of a medicament for raising an immune response in a mammal. The use of a 'NadA' protein, a '741' protein, a '936' protein, a '953' protein, and a '287' protein (and other optional antigens) in the manufacture of a medicament for raising an immune response in a mammal is also disclosed. The medicament is preferably a vaccine.

[0074] Also disclosed is a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies. The method may raise a booster response.

[0075] 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); 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.

[0076] These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Neisseria* (e.g. meningitis, septicaemia, bacteremia, gonorrhoea etc.). The prevention and/or treatment of bacterial or meningococcal meningitis is preferred.

[0077] One way of checking efficacy of therapeutic treatment involves monitoring Neisserial infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the five basic 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 [27]) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT) of total and high-avidity anti-capsule IgG. 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.

[0078] Preferred compositions of the invention can confer an antibody titre in a patient that is superior to the criterion for seroprotection for each antigenic component for an acceptable percentage of human subjects. Antigens with an associated antibody titre above which a host is considered to be seroconverted against the antigen are well known, and such titres are published by organisations such as WHO. Preferably more than 80% of a statistically significant sample of subjects is seroconverted, more preferably more than 90%, still more preferably more than 93% and most preferably 96-100%.

[0079] 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 rectal, oral, vaginal, topical, transdermal, intranasal, ocular, aural, pulmonary or other mucosal administration. 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.

[0080] The invention may be used to elicit systemic and/or mucosal immunity.

[0081] 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.

[0082] Neisserial infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (e.g. a lyophilised composition). The composition may be prepared for topical administration e.g. as an ointment, cream or powder. The composition may be prepared for oral administration e.g. as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration e.g. as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration e.g. as spray, drops, gel or powder [e.g. refs 28 & 29]. Success with nasal administration of pneumococcal saccharides [30,31], pneumococcal polypeptides [32], Hib saccharides [33], MenC saccharides [34], and mixtures of Hib and MenC saccharide conjugates [35] has been reported.

[0083] 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, and a typical quantity of each meningococcal saccharide antigen per dose is between 1µg and 20µg e.g. about 1µg, about 2.5µg, about 4µg, about 5µg, or about 10µg (expressed as saccharide).

Further non-antigen components of the composition

[0084] The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose [36], trehalose [37], lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable excipients is available in reference 38.

[0085] Compositions of the invention may include an antimicrobial, particularly when packaged in multiple dose format.

[0086] 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%.

[0087] 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.

[0088] Compositions of the invention will generally include a buffer. A phosphate buffer is typical.

[0089] Compositions of the invention may comprise a sugar alcohol (e.g. mannitol) or a disaccharide (e.g. sucrose or trehalose) e.g. at around 15-30 mg/ml (e.g. 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised material. The pH of a composition for lyophilisation may be adjusted to around 6.1 prior to lyophilisation.

[0090] Vaccines of the invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

[0091] Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (e.g. oxyhydroxides), phosphates (e.g. hydroxyphosphates, orthophosphates), sulphates, etc. [e.g. see chapters 8 & 9 of ref. 39], or mixtures of different mineral compounds, with the compounds taking any suitable form (e.g. gel, crystalline, amorphous, etc.), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [40].

[0092] Aluminium phosphates are particularly preferred, particularly in compositions which include a *H.influenzae* saccharide antigen, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at $0.6 \text{ mg Al}^{3+}/\text{ml}$. Adsorption with a low dose of aluminium phosphate may be used e.g. between 50 and $100 \mu\text{g Al}^{3+}$ per conjugate per dose. Where there is more than one conjugate in a composition, not all conjugates need to be adsorbed.

B. Oil Emulsions

[0093] Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 39; see also ref. 41] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 39]

[0094] Saponin formulations may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officinalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

[0095] Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 42. Saponin formulations may also comprise a sterol, such as cholesterol [43].

[0096] Combinations of saponins and cholesterol can be used to form unique particles called immunostimulating complexes (ISCOMs) [chapter 23 of ref. 39]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA & QHC. ISCOMs are further described in refs. 43-45. Optionally, the ISCOMS may be devoid of additional detergent [46].

[0097] A review of the development of saponin based adjuvants can be found in refs. 47 & 48.

D. Virosomes and virus-like particles

[0098] Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Q β -phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 49-54. Virosomes are discussed further in, for example, ref. 55

E. Bacterial or microbial derivatives

[0099] Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

[0100] Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 56. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 μ m membrane [56]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives e.g. RC-529 [57,58].

[0101] Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 59 & 60.

[0102] Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

[0103] The CpG's can include nucleotide modifications/analogues such as phosphorothioate modifications and can be double-stranded or single-stranded. References 61, 62 and 63 disclose possible analog substitutions e.g. replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG oligonucleotides is further discussed in refs. 64-69.

[0104] The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [70]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such as a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 71-73. Preferably, the CpG is a CpG-A ODN.

[0105] Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 70 & 74-76.

[0106] Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 77 and as parenteral adjuvants in ref. 78. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and detoxified derivatives thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 79-86. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 87.

F. Human immunomodulators

[0107] Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [88], *etc.*) [89], interferons (e.g. interferon- γ), macrophage colony stimulating factor, and tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

[0108] Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [90] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [91].

H. Microparticles

[0109] Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150 μ m in diameter, more preferably ~200nm to ~30 μ m in diameter, and most preferably ~500nm to ~10 μ m in diameter) formed from materials that are biodegradable and non-toxic (e.g. a poly(α -hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (e.g. with SDS) or a positively-charged surface (e.g. with a cationic detergent, such as CTAB).

I. Liposomes (chapters 13 & 14 of ref. 39)

[0110] Examples of liposome formulations suitable for use as adjuvants are described in refs. 92-94.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

[0111] Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [95]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [96] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [97]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Polyphosphazene (PCPP)

[0112] PCPP formulations are described, for example, in refs. 98 and 99.

L. Muramyl peptides

[0113] Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

[0114] Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (e.g. "Resiquimod 3M"), described further in refs. 100 and 101.

[0115] The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example,

the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [102]; (2) a saponin (e.g. QS21) + a non-toxic LPS derivative (e.g. 3dMPL) [103]; (3) a saponin (e.g. QS21) + a non-toxic LPS derivative (e.g. 3dMPL) + a cholesterol; (4) a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) [104]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [105]; (6) SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

[0116] Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 39.

[0117] The use of an aluminium hydroxide or aluminium phosphate adjuvant is particularly preferred, and antigens are generally adsorbed to these salts. Aluminium hydroxide is preferably avoided as an adjuvant if the composition includes a H1b antigen. Where an aluminium phosphate is used and desired not to adsorb an antigen to the adjuvant, this is favoured by including free phosphate ions in solution (e.g. by the use of a phosphate buffer). Prevention of adsorption can also be achieved by selecting the correct pH during antigen/adjuvant mixing, an adjuvant with an appropriate point of zero charge, and an appropriate order of mixing for different antigens in a composition [106].

[0118] Calcium phosphate is another preferred adjuvant.

Further antigens

[0119] Compositions of the invention contain five basic meningococcal protein antigens. They may also include further antigens, although it may contain no meningococcal protein antigens other than the five basic antigens. Further antigens for inclusion may be, for example:

- a saccharide antigen from *Haemophilus influenzae* B.
- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 107 from serogroup C or the oligosaccharides of ref. 108.
- a saccharide antigen from *Streptococcus pneumoniae* [e.g. 155, 156 157].
- an antigen from hepatitis A virus, such as inactivated virus [e.g. 109, 110].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [e.g. 110, 111].
- a diphtheria antigen, such as a diphtheria toxoid [e.g. chapter 3 of ref. 112] e.g. the CRM₁₉₇ mutant [e.g. 113].
- a tetanus antigen, such as a tetanus toxoid [e.g. chapter 4 of ref. 112].
- 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 [e.g. refs. 114 & 115]. Cellular pertussis antigen may be used.
- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 4, 116, 117, 118 etc.
- polio antigen(s) [e.g. 119, 120] such as OPV or, preferably, IPV.

[0120] The composition may comprise one or more of these further antigens. Antigens will typically be present at a concentration of at least 1µg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen. It is preferred that the protective efficacy of individual saccharide antigens is not removed by combining them, although actual immunogenicity (e.g. ELISA titres) may be reduced.

[0121] 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. Such DTP combinations can be used to reconstitute lyophilised conjugates.

[0122] Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity (see below).

[0123] Toxic protein antigens may be detoxified where necessary (e.g. detoxification of pertussis toxin by chemical and/or genetic means [115]).

[0124] As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used [e.g. refs. 121 to 129]. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA e.g. in the form of a plasmid) that encodes the protein. Similarly, compositions of the invention may comprise proteins which mimic saccharide antigens e.g. mimotopes [130] or anti-idiotypic antibodies. These may replace individual saccharide components, or may supplement them. As an example, the vaccine may comprise a peptide mimic of the MenC [131] or the MenA [132] capsular polysaccharide in place of the saccharide itself.

[0125] Particularly preferred compositions of the invention include one, two or three of: (a) saccharide antigens from meningococcus serogroups Y, W135, C and (optionally) A; (b) a saccharide antigen from *Haemophilus influenzae* type B; and/or (c) an antigen from *Streptococcus pneumoniae*. A composition comprising the serogroup B antigens and a Hib conjugate is particularly preferred.

Meningococcus serogroups Y, W135, C and (optionally) A

[0126] As mentioned above, polysaccharide vaccines against serogroups A, C, W135 & Y has been known for many years. These vaccines (MENGEVAX ACWY™ and MENOMUNE™) are based on the organisms' capsular polysaccharides and, although they are effective in adolescents and adults, they give a poor immune response and short duration of protection, and they cannot be used in infants.

[0127] In contrast to the unconjugated polysaccharide antigens in these vaccines, the recently-approved serogroup C vaccines (Menjugate™ [133,107], Meningitec™ and NeisVac-C™) include conjugated saccharides. Menjugate™ and Meningitec™ have oligosaccharide antigens conjugated to a CRM₁₉₇ carrier, whereas NeisVac-C™ uses the complete polysaccharide (de-O-acetylated) conjugated to a tetanus toxoid carrier.

[0128] Compositions of the present invention preferably include capsular saccharide antigens from one or more of meningococcus serogroups Y, W135, C and (optionally) A, wherein the antigens are conjugated to carrier protein(s) and/or are oligosaccharides.

[0129] A typical quantity of each meningococcal saccharide antigen per dose is between 1µg and 20µg e.g. about 1µg, about 2.5µg, about 4µg, about 5µg, or about 10µg (expressed as saccharide).

[0130] Where a mixture comprises capsular saccharides from both serogroups A and C, the ratio (w/w) of MenA saccharide:MenC saccharide may be greater than 1 (e.g. 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Where a mixture comprises capsular saccharides from serogroup Y and one or both of serogroups C and W135, the ratio (w/w) of MenY saccharide:MenW135 saccharide may be greater than 1 (e.g. 2:1, 3:1, 4:1, 5:1, 10:1 or higher) and/or that the ratio (w/w) of MenY saccharide:MenC saccharide may be less than 1 (e.g. 1:2, 1:3, 1:4, 1:5, or lower). Preferred ratios (w/w) for saccharides from serogroups A:C:W135:Y are: 1:1:1:1; 1:1:1:2; 2:1:1:1; 4:2:1:1; 8:4:2:1; 4:2:1:2; 8:4:1:2; 4:2:2:1; 2:2:1:1; 4:4:2:1; 2:2:1:2; 4:4:1:2; and 2:2:2:1. Preferred ratios (w/w) for saccharides from serogroups C:W135:Y are: 1:1:1; 1:1:2; 1:1:1; 2:1:1; 4:2:1; 2:1:2; 4:1:2; 2:2:1; and 2:1:1. Using a substantially equal mass of each saccharide is preferred.

[0131] Capsular saccharides will generally be used in the form of oligosaccharides. These are conveniently formed by fragmentation of purified capsular polysaccharide (e.g. by hydrolysis), which will usually be followed by purification of the fragments of the desired size.

[0132] Fragmentation of polysaccharides is preferably performed to give a final average degree of polymerisation (DP) in the oligosaccharide of less than 30 (e.g. between 10 and 20, preferably around 10 for serogroup A; between 15 and 25 for serogroups W135 and Y, preferably around 15-20; between 12 and 22 for serogroup C; etc.). DP can conveniently be measured by ion exchange chromatography or by colorimetric assays [134].

[0133] If hydrolysis is performed, the hydrolysate will generally be sized in order to remove short-length oligosaccharides [135]. This can be achieved in various ways, such as ultrafiltration followed by ion-exchange chromatography. Oligosaccharides with a degree of polymerisation of less than or equal to about 6 are preferably removed for serogroup A, and those less than around 4 are preferably removed for serogroups W135 and Y.

[0134] Preferred MenC saccharide antigens are disclosed in reference 133, as used in Menjugate™.

[0135] The saccharide antigen may be chemically modified. This is particularly useful for reducing hydrolysis for serogroup A [136; see below]. De-O-acetylation of meningococcal saccharides can be performed. For oligosaccharides, modification may take place before or after depolymerisation.

[0136] Where a composition of the invention includes a MenA saccharide antigen, the antigen is preferably a modified saccharide in which one or more of the hydroxyl groups on the native saccharide has/have been replaced by a blocking group [136]. This modification improves resistance to hydrolysis, and means that the serogroup A antigen can be stored and used in a liquid formulation rather than requiring lyophilisation.

[0137] The number of monosaccharide units having blocking groups can vary. For example, all or substantially all the monosaccharide units may have blocking groups. Alternatively, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the monosaccharide units may have blocking groups. At least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 monosaccharide units may have blocking groups.

[0138] Likewise, the number of blocking groups on a monosaccharide unit may vary. For example, the number of blocking groups on a monosaccharide unit may be 1 or 2. The blocking group will generally be at the 4 position and/or 3-position of the monosaccharide units.

[0139] The terminal monosaccharide unit may or may not have a blocking group instead of its native hydroxyl. It is preferred to retain a free anomeric hydroxyl group on a terminal monosaccharide unit in order to provide a handle for further reactions (e.g. conjugation). Anomeric hydroxyl groups can be converted to amino groups (-NH₂ or -NH-E, where E is a nitrogen protecting group) by reductive amination (using, for example, NaBH₃CN/NH₄Cl), and can then be regenerated after other hydroxyl groups have been converted to blocking groups.

[0140] Blocking groups to replace hydroxyl groups may be directly accessible via a derivatizing reaction of the hydroxyl group *i.e.* by replacing the hydrogen atom of the hydroxyl group with another group. Suitable derivatives of hydroxyl groups which act as blocking groups are, for example, carbamates, sulfonates, carbonates, esters, ethers (e.g. silyl ethers or alkyl ethers) and acetals. Some specific examples of such blocking groups are allyl, Aloc, benzyl, BOM, t-butyl, trityl, TBS, TBDPS, TES, TMS, TIPS, PMB, MEM, MOM, MTM, THP, *etc.* Other blocking groups that are not directly accessible and which completely replace the hydroxyl group include C₁₋₁₂ alkyl, C₃₋₁₂ alkyl, C₅₋₁₂ aryl, C₅₋₁₂ aryl-C₁₋₆ alkyl, NR¹R² (R¹ and R² are defined in the following paragraph), H, F, Cl, Br, CO₂H, CO₂(C₁₋₆ alkyl), CN, CF₃, CCl₃, *etc.* Preferred blocking groups are electron-withdrawing groups.

[0141] Preferred blocking groups are of the formula: -O-X-Y or -OR³ wherein: X is C(O), S(O) or SO₂; Y is C₁₋₁₂ alkyl, C₁₋₁₂ alkoxy, C₃₋₁₂ cycloalkyl, C₅₋₁₂ aryl or C₅₋₁₂ aryl-C₁₋₆ alkyl, each of which may optionally be substituted with 1, 2 or 3 groups independently selected from F, Cl, Br, CO₂H, CO₂(C₁₋₆ alkyl), CN, CF₃ or CCl₃; or Y is NR¹R²; R¹ and R² are independently selected from H, C₁₋₁₂ alkyl, C₃₋₁₂ cycloalkyl, C₅₋₁₂ aryl, C₅₋₁₂ aryl-C₁₋₆ alkyl; or R¹ and R² may be joined to form a C₃₋₁₂ saturated heterocyclic group; R³ is C₁₋₁₂ alkyl or C₃₋₁₂ cycloalkyl, each of which may optionally be substituted with 1, 2 or 3 groups independently selected from F, Cl, Br, CO₂(C₁₋₆ alkyl), CN, CF₃ or CCl₃; or R³ is C₅₋₁₂ aryl or C₅₋₁₂ aryl-C₁₋₆ alkyl, each of which may optionally be substituted with 1, 2, 3, 4 or 5 groups selected from F, Cl, Br, CO₂H, CO₂(C₁₋₆ alkyl), CN, CF₃ or CCl₃. When R³ is C₁₋₁₂ alkyl or C₃₋₁₂ cycloalkyl, it is typically substituted with 1, 2 or 3 groups as defined above. When R¹ and R² are joined to form a C₃₋₁₂ saturated heterocyclic group, it is meant that R¹ and R² together with the nitrogen atom form a saturated heterocyclic group containing any number of carbon atoms between 3 and 12 (e.g. C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂). The heterocyclic group may contain 1 or 2 heteroatoms (such as N, O or S) other than the nitrogen atom. Examples of C₃₋₁₂ saturated heterocyclic groups are pyrrolidinyl, piperidinyl, morpholinyl, piperazinyl, imidazolidinyl, azetidiny and aziridinyl.

[0142] Blocking groups -O-X-Y and -OR³ can be prepared from -OH groups by standard derivatizing procedures, such as reaction of the hydroxyl group with an acyl halide, alkyl halide, sulfonyl halide, *etc.* Hence, the oxygen atom in -O-X-Y is preferably the oxygen atom of the hydroxyl group, while the -X-Y group in -O-X-Y preferably replaces the hydrogen atom of the hydroxyl group.

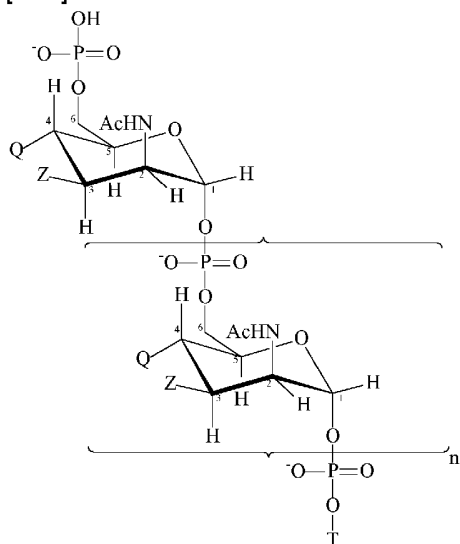
[0143] Alternatively, the blocking groups may be accessible via a substitution reaction, such as a Mitsunobu-type substitution. These and other methods of preparing blocking groups from hydroxyl groups are well known.

[0144] More preferably, the blocking group is $-\text{OC}(\text{O})\text{CF}_3$ [137], or a carbamate group $-\text{OC}(\text{O})\text{NR}^1\text{R}^2$, where R^1 and R^2 are independently selected from C_{1-6} alkyl. More preferably, R^1 and R^2 are both methyl *i.e.* the blocking group is $-\text{OC}(\text{O})\text{NMe}_2$. Carbamate blocking groups have a stabilizing effect on the glycosidic bond and may be prepared under mild conditions.

[0145] Preferred modified MenA saccharides contain n monosaccharide units, where at least $h\%$ of the monosaccharide units do not have -OH groups at both of positions 3 and 4. The value of h is 24 or more (e.g. 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 98, 99 or 100) and is preferably 50 or more. The absent -OH groups are preferably blocking groups as defined above.

[0146] Other preferred modified MenA saccharides comprise monosaccharide units, wherein at least s of the monosaccharide units do not have -OH at the 3 position and do not have -OH at the 4 position. The value of s is at least 1 (e.g. 2, 3, 4, 5, 6, 7, 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). The absent -OH groups are preferably blocking groups as defined above.

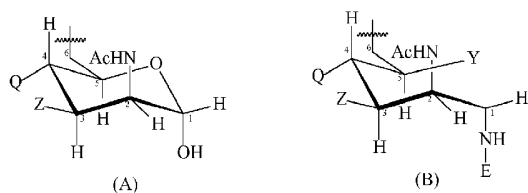
[0147] Suitable modified MenA saccharides for use with the invention have the formula:



wherein

n is an integer from 1 to 100 (preferably an integer from 15 to 25);

T is of the formula (A) or (B):



each Z group is independently selected from OH or a blocking group as defined above; and each Q group is independently selected from OH or a blocking group as defined above;

Y is selected from OH or a blocking group as defined above;

E is H or a nitrogen protecting group;

and wherein more than about 7% (e.g. 8%, 9%, 10% or more) of the Q groups are blocking groups.

[0148] Each of the $n+2$ Z groups may be the same or different from each other. Likewise, each of the $n+2$ Q groups may be the

same or different from each other. All the Z groups may be OH. Alternatively, at least 10%, 20, 30%, 40%, 50% or 60% of the Z groups may be OAc. Preferably, about 70% of the Z groups are OAc, with the remainder of the Z groups being OH or blocking groups as defined above. At least about 7% of Q groups are blocking groups. Preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or even 100% of the Q groups are blocking groups.

[0149] Preferred compositions of the invention can be stored for 28 days at 37°C and, after that period, less than $f\%$ of the initial total amount of conjugated MenA saccharide will be unconjugated, where f is 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or lower.

[0150] Meningococcal capsular polysaccharides are typically prepared by a process comprising the steps of polysaccharide precipitation (e.g. using a cationic detergent), ethanol fractionation, cold phenol extraction (to remove protein) and ultracentrifugation (to remove LPS) [e.g. ref. 138]. A more preferred process [108], however, involves polysaccharide precipitation followed by solubilisation of the precipitated polysaccharide using a lower alcohol. Precipitation can be achieved using a cationic detergent such as tetrabutylammonium and cetyltrimethylammonium salts (e.g. the bromide salts), or hexadimethrine bromide and myristyltrimethylammonium salts. Cetyltrimethylammonium bromide ('CTAB') is particularly preferred [139]. Solubilisation of the precipitated material can be achieved using a lower alcohol such as methanol, propan-1-ol, propan-2-ol, butan-1-ol, butan-2-ol, 2-methyl-propan-1-ol, 2-methyl-propan-2-ol, diols, etc., but ethanol is particularly suitable for solubilising CTAB-polysaccharide complexes. Ethanol is preferably added to the precipitated polysaccharide to give a final concentration (based on total content of ethanol and water) of between 50% and 95%.

[0151] After re-solubilisation, the polysaccharide may be further treated to remove contaminants. This is particularly important in situations where even minor contamination is not acceptable (e.g. for human vaccine production). This will typically involve one or more steps of filtration e.g. depth filtration, filtration through activated carbon may be used, size filtration and/or ultrafiltration. Once filtered to remove contaminants, the polysaccharide may be precipitated for further treatment and/or processing. This can be conveniently achieved by exchanging cations (e.g. by the addition of calcium or sodium salts).

[0152] As an alternative to purification, capsular saccharides of the present invention may be obtained by total or partial synthesis e.g. Hib synthesis is disclosed in ref. 140, and MenA synthesis in ref. 141.

[0153] Compositions of the invention comprise capsular saccharides from at least two serogroups of *N.meningitidis*. The saccharides are preferably prepared separately (including any fragmentation, conjugation, modification, etc.) and then admixed to give a composition of the invention.

[0154] Where the composition comprises capsular saccharide from serogroup A, however, it is preferred that the serogroup A saccharide is not combined with the other saccharide(s) until shortly before use, in order to minimise the potential for hydrolysis. This can conveniently be achieved by having the serogroup A component (typically together with appropriate excipients) in lyophilised form and the other serogroup component(s) in liquid form (also with appropriate excipients), with the liquid components being used to reconstitute the lyophilised MenA component when ready for use. Where an aluminium salt adjuvant is used, it is preferred to include the adjuvant in the vial containing the with the liquid vaccine, and to lyophilise the MenA component without adjuvant.

[0155] A composition of the invention may thus be prepared from a kit comprising: (a) capsular saccharide from *N.meningitidis* serogroup A, in lyophilised form; and (b) the further antigens from the composition, in liquid form.

[0156] Within each dose, the amount of an individual saccharide antigen will generally be between 1-50 µg (measured as mass of saccharide), with about 2.5µg, 5µg or 10 µg of each being preferred. With A:C:W135:Y weight ratios of 1:1:1:1; 1:1:1:2; 2:1:1:1; 4:2:1:1; 8:4:2:1; 4:2:1:2; 8:4:1:2; 4:2:2:1; 2:2:1:1; 4:4:2:1; 2:2:1:2; 4:4:1:2; and 2:2:2:1, therefore, the amount represented by the figure 1 is preferably about 2.5µg, 5µg or 10 µg. For a 1:1:1:1 ratio A:C:W:Y composition and a 10µg per saccharide, therefore, 40 µg saccharide is administered per dose. Preferred compositions have about the following µg saccharide per dose:

A	10	0	0	0	10	5	2.5
C	10	10	5	2.5	5	5	2.5
W135	10	10	5	2.5	5	5	2.5
Y	10	10	5	2.5	5	5	2.5

Preferred compositions of the invention comprise less than 50 µg meningococcal saccharide per dose. Other preferred compositions comprise ≤40 µg meningococcal saccharide per dose. Other preferred compositions comprise ≤30 µg meningococcal saccharide per dose. Other preferred compositions comprise ≤25 µg meningococcal saccharide per dose. Other

preferred compositions comprise ≤ 20 μg meningococcal saccharide per dose. Other preferred compositions comprise ≤ 10 μg meningococcal saccharide per dose but, ideally, compositions of the invention comprise at least 10 μg meningococcal saccharide per dose.

[0157] The Menjugate™ and NeisVac™ MenC conjugates use a hydroxide adjuvant, whereas Meningitec™ uses a phosphate. It is possible in compositions of the invention to adsorb some antigens to an aluminium hydroxide but to have other antigens in association with an aluminium phosphate. For tetravalent serogroup combinations, for example, the following permutations are available:

Serogroup	Aluminium salt (H = a hydroxide; P = a phosphate)															
A	P	H	P	H	H	H	P	P	P	H	H	H	P	P	P	H
C	P	H	H	P	H	H	P	H	H	P	P	H	P	H	P	P
W135	P	H	H	H	P	H	H	P	H	H	P	P	P	P	H	P
Y	P	H	H	H	H	P	H	H	P	P	H	P	H	P	P	P

[0158] For trivalent *N.meningitidis* serogroup combinations, the following permutations are available:

Serogroup	Aluminium salt (H = a hydroxide; P = a phosphate)							
C	P	H	H	H	P	P	P	H
W135	P	H	H	P	H	P	H	P
Y	P	H	P	H	H	H	P	P

Haemophilus influenzae type B

[0159] Where the composition includes a *H.influenzae* type B antigen, it will typically be a Hib capsular saccharide antigen. Saccharide antigens from *H.influenzae* b are well known.

[0160] Advantageously, the Hib saccharide is covalently conjugated to a carrier protein, in order to enhance its immunogenicity, especially in children. The preparation of polysaccharide conjugates in general, and of the Hib capsular polysaccharide in particular, is well documented [e.g. references 142 to 150 *etc.*]. The invention may use any suitable Hib conjugate. Suitable carrier proteins are described below, and preferred carriers for Hib saccharides are CRM₁₉₇ ('HbOC'), tetanus toxoid ('PRP-T') and the outer membrane complex of *N.meningitidis* ('PRP-OMP').

[0161] The saccharide moiety of the conjugate may be a polysaccharide (e.g. full-length polyribosylribitol phosphate (PRP)), but it is preferred to hydrolyse polysaccharides to form oligosaccharides (e.g. MW from ~1 to ~5 kDa).

[0162] A preferred conjugate comprises a Hib oligosaccharide covalently linked to CRM₁₉₇ via an adipic acid linker [151, 152]. Tetanus toxoid is also a preferred carrier.

[0163] Administration of the Hib antigen preferably results in an anti-PRP antibody concentration of $\geq 0.15 \mu\text{g/ml}$, and more preferably $\geq 1 \mu\text{g/ml}$.

[0164] Compositions of the invention may comprise more than one Hib antigen.

[0165] Where a composition includes a Hib saccharide antigen, it is preferred that it does not also include an aluminium hydroxide adjuvant. If the composition includes an aluminium phosphate adjuvant then the Hib antigen may be adsorbed to the adjuvant [153] or it may be non-adsorbed [154].

[0166] Hib antigens may be lyophilised e.g. together with meningococcal antigens.

Streptococcus pneumoniae

[0167] Where the composition includes a *S.pneumoniae* antigen, it will typically be a capsular saccharide antigen which is preferably conjugated to a carrier protein [e.g. refs. 155 to 157]. It is preferred to include saccharides from more than one serotype of *S.pneumoniae*. For example, mixtures of polysaccharides from 23 different serotype are widely used, as are conjugate vaccines with polysaccharides from between 5 and 11 different serotypes [158]. For example, PreVNar™ [159] contains antigens from seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) with each saccharide individually conjugated to CRM₁₉₇ by reductive amination, with 2µg of each saccharide per 0.5ml dose (4µg of serotype 6B), and with conjugates adsorbed on an aluminium phosphate adjuvant. Compositions of the invention preferably include at least serotypes 6B, 14, 19F and 23F. Conjugates may be adsorbed onto an aluminium phosphate.

[0168] As an alternative to using saccharide antigens from pneumococcus, the composition may include one or more polypeptide antigens. Genome sequences for several strains of pneumococcus are available [160,161] and can be subjected to reverse vaccinology [162-165] to identify suitable polypeptide antigens [166,167]. For example, the composition may include one or more of the following antigens: PhtA, PhtD, PhtB, PhtE, SpsA, LytB, LytC, LytA, Sp125, Sp101, Sp128, Sp130 and Sp130, as defined in reference 168. The composition may include more than one (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14) of these antigens.

[0169] In some embodiments, the composition may include both saccharide and polypeptide antigens from pneumococcus. These may be used in simple admixture, or the pneumococcal saccharide antigen may be conjugated to a pneumococcal protein. Suitable carrier proteins for such embodiments include the antigens listed in the previous paragraph [168].

[0170] Pneumococcal antigens may be lyophilised e.g. together with meningococcal and/or Hib antigens.

Covalent conjugation

[0171] Capsular saccharides in compositions of the invention will usually be conjugated to carrier protein(s). In general, conjugation enhances the immunogenicity of saccharides as it converts them from T-independent antigens to T-dependent antigens, thus allowing priming for immunological memory. Conjugation is particularly useful for paediatric vaccines and is a well known technique [e.g. reviewed in refs. 169 and 142-150].

[0172] Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria toxoid or tetanus toxoid. The CRM₁₉₇ diphtheria toxoid [170-172] is particularly preferred. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [173], synthetic peptides [174,175], heat shock proteins [176,177], pertussis proteins [178,179], cytokines [180], lymphokines [180], hormones [180], growth factors [180], artificial proteins comprising multiple human CD4⁺ T cell epitopes from various pathogen-derived antigens [181], protein D from *H.influenzae* [182,183], pneumococcal surface protein PspA [184], iron-uptake proteins [185], toxin A or B from *C.difficile* [186], etc. Preferred carriers are diphtheria toxoid, tetanus toxoid, *H.influenzae* protein D, and CRM₁₉₇.

[0173] Within a composition of the invention, it is possible to use more than one carrier protein e.g. to reduce the risk of carrier suppression. Thus different carrier proteins can be used for different serogroups e.g. serogroup A saccharides might be conjugated to CRM₁₉₇ while serogroup C saccharides might be conjugated to tetanus toxoid. It is also possible to use more than one carrier protein for a particular saccharide antigen e.g. serogroup A saccharides might be in two groups, with some conjugated to CRM₁₉₇ and others conjugated to tetanus toxoid. In general, however, it is preferred to use the same carrier protein for all saccharides.

[0174] A single carrier protein might carry more than one saccharide antigen [187]. For example, a single carrier protein might have conjugated to it saccharides from serogroups A and C. To achieve this goal, saccharides can be mixed prior to the conjugation reaction. In general, however, it is preferred to have separate conjugates for each serogroup.

[0175] Conjugates with a saccharide:protein ratio (w/w) of between 1:5 (i. e. excess protein) and 5:1 (i. e. excess saccharide) are preferred. Ratios between 1:2 and 5:1 are preferred, as are ratios between 1:1.25 and 1:2.5 are more preferred. Excess carrier protein may be preferred for MenA and MenC.

[0176] Conjugates may be used in conjunction with free carrier protein [188]. When a given carrier protein is present in both free and conjugated form in a composition of the invention, the unconjugated form is preferably no more than 5% of the total amount of the carrier protein in the composition as a whole, and more preferably present at less than 2% by weight.

[0177] Any suitable conjugation reaction can be used, with any suitable linker where necessary.

[0178] The saccharide will typically be activated or functionalised prior to conjugation. Activation may involve, for example, cyanylating reagents such as CDAP (e.g. 1-cyano-4-dimethylamino pyridinium tetrafluoroborate [189,190,etc.]). Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S-NHS, EDC, TSTU; see also the introduction to reference 148).

[0179] Linkages via a linker group may be made using any known procedure, for example, the procedures described in references 191 and 192. One type of linkage involves reductive amination of the polysaccharide, coupling the resulting amino group with one end of an adipic acid linker group, and then coupling a protein to the other end of the adipic acid linker group [146,193,194]. Other linkers include B-propionamido [195], nitrophenyl-ethylamine [196], haloacyl halides [197], glycosidic linkages [198], 6-aminocaproic acid [199], ADH [200], C₄ to C₁₂ moieties [201] etc. As an alternative to using a linker, direct linkage can be used. Direct linkages to the protein may comprise oxidation of the polysaccharide followed by reductive amination with the protein, as described in, for example, references 202 and 203.

[0180] A process involving the introduction of amino groups into the saccharide (e.g. by replacing terminal =O groups with -NH₂) followed by derivatisation with an adipic diester (e.g. adipic acid N-hydroxysuccinimido diester) and reaction with carrier protein is preferred. Another preferred reaction uses CDAP activation with a protein D carrier e.g. for MenA or MenC.

[0181] After conjugation, free and conjugated saccharides can be separated. There are many suitable methods, including hydrophobic chromatography, tangential ultrafiltration, diafiltration etc. [see also refs. 204 & 205, etc.].

[0182] Where the composition of the invention includes a conjugated oligosaccharide, it is preferred that oligosaccharide preparation precedes conjugation.

Further and alternative serogroup B polypeptides antigens

[0183] The invention provides a composition as defined in the claims which, after administration to a subject, is able to induce an antibody response in that subject, wherein the antibody response is bactericidal against two or three of hypervirulent lineages A4, ET-5 and lineage 3 of *N.meningitidis* serogroup B.

[0184] Although NadA, 741, 936, 953 and 287 are preferred antigens for achieving this broad protection, other MenB polypeptide antigens which may be included in compositions of the invention (in combination with the five basic antigens as defined in the claims) include those comprising one of the following amino acid sequences: SEQ ID NO:650 from ref. 8; SEQ ID NO:878 from ref. 8; SEQ ID NO:884 from ref. 8; SEQ ID NO:4 from ref. 9; SEQ ID NO:598 from ref. 10; SEQ ID NO:818 from ref. 10; SEQ ID NO:864 from ref. 10; SEQ ID NO:866 from ref. 10; SEQ ID NO:1196 from ref. 10; SEQ ID NO:1272 from ref. 10; SEQ ID NO:1274 from ref. 10; SEQ ID NO:1640 from ref. 10; SEQ ID NO:1788 from ref. 10; SEQ ID NO:2288 from ref. 10; SEQ ID NO:2466 from ref. 10; SEQ ID NO:2554 from ref. 10; SEQ ID NO:2576 from ref. 10; SEQ ID NO:2606 from ref. 10; SEQ ID NO:2608 from ref. 10; SEQ ID NO:2616 from ref. 10; SEQ ID NO:2668 from ref. 10; SEQ ID NO:2780 from ref. 10; SEQ ID NO:2932 from ref. 10; SEQ ID NO:2958 from ref. 10; SEQ ID NO:2970 from ref. 10; SEQ ID NO:2988 from ref. 10, or a polypeptide comprising an amino acid sequence which: (a) has 50% or more identity (e.g. 60%, 70%, 80%, 90%, 95%, 99% or more) to said sequences; and/or (b) comprises a fragment of at least *n* consecutive amino acids from said sequences, wherein *n* is 7 or more (eg. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments for (b) comprise an epitope from the relevant sequence. More than one (e.g. 2, 3, 4, 5, 6) of these polypeptides may be included.

General

[0185] The term "comprising" means "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.

[0186] The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

[0187] 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.

[0188] 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 reference 206. 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 taught in reference 207.

[0189] The term "alkyl" refers to alkyl groups in both straight and branched forms. The alkyl group may be interrupted with 1, 2 or 3 heteroatoms selected from -O-, -NH- or -S-. The alkyl group may also be interrupted with 1, 2 or 3 double and/or triple bonds. However, the term "alkyl" usually refers to alkyl groups having no heteroatom interruptions or double or triple bond interruptions. Where reference is made to C₁₋₁₂ alkyl, it is meant the alkyl group may contain any number of carbon atoms between 1 and 12 (e.g. C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂). Similarly, where reference is made to C₁₋₆ alkyl, it is meant the alkyl group may contain any number of carbon atoms between 1 and 6 (e.g. C₁, C₂, C₃, C₄, C₅, C₆).

[0190] The term "cycloalkyl" includes cycloalkyl, polycycloalkyl, and cycloalkenyl groups, as well as combinations of these with alkyl groups, such as cycloalkylalkyl groups. The cycloalkyl group may be interrupted with 1, 2 or 3 heteroatoms selected from -O-, -NH- or -S-. However, the term "cycloalkyl" usually refers to cycloalkyl groups having no heteroatom interruptions. Examples of cycloalkyl groups include cyclopentyl, cyclohexyl, cyclohexenyl, cyclohexylmethyl and adamantyl groups. Where reference is made to C₃₋₁₂ cycloalkyl, it is meant that the cycloalkyl group may contain any number of carbon atoms between 3 and 12 (e.g. C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂).

[0191] The term "aryl" refers to an aromatic group, such as phenyl or naphthyl. Where reference is made to C₅₋₁₂ aryl, it is meant that the aryl group may contain any number of carbon atoms between 5 and 12 (e.g. C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂).

[0192] The term "C₅₋₁₂ aryl-C₁₋₆ alkyl" refers to groups such as benzyl, phenylethyl and naphthylmethyl.

[0193] Nitrogen protecting groups include silyl groups (such as TMS, TES, TBS, TIPS), acyl derivatives (such as phthalimides, trifluoroacetamides, methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl (Boc), benzyloxycarbonyl (Z or Cbz), 9-fluorenylmethoxycarbonyl (Fmoc), 2-(trimethylsilyl)ethoxy carbonyl, 2,2,2-trichloroethoxycarbonyl (Troc)), sulfonyl derivatives (such as β -trimethylsilyl ethanesulfonyl (SES)), sulfenyl derivatives, C₁₋₁₂ alkyl, benzyl, benzhydryl, trityl, 9-phenylfluorenyl etc. A preferred nitrogen protecting group is Fmoc.

[0194] Sequences included to facilitate cloning or purification, etc., do not necessarily contribute to the invention and may be omitted or removed.

[0195] It will be appreciated that sugar rings can exist in open and closed form and that, whilst closed forms are shown in structural formulae herein, open forms are also encompassed by the invention.

MODES FOR CARRYING OUT THE INVENTION

ΔG287-953 hybrid protein

[0196] DNA encoding protein 287 from meningococcal serogroup B strain 394/98 and protein 953 from meningococcal serogroup B strain 2996 were digested and ligated, together with a short linker sequence, to give a plasmid encoding amino acid sequence SEQ ID 7. The plasmid was transfected into *E. coli* and bacteria were grown to express the protein.

[0197] After adequate growth, bacteria were harvested and the protein was purified. From culture, bacteria were centrifuged and the pellet was homogenized in the presence of 50 mM acetate buffer (pH 5) with a pellet:buffer volume ratio of 1: 8. Lysis was performed using a high pressure homogenizer (AVESTIN, 4 cycles at 14000 psi). After lysis, urea was added at final concentration of 5M, followed by agitation for 1 hour at room temperature. The pH was reduced from 6 to 5 using 200 mM acetate buffer (pH 4) + 5 M urea. The mixture was centrifuged at 16800g for 60 minutes at 2-8°C. The supernatant was collected and filtered by SARTOBRA P (0.45-0.22µm SARTORIUS).

[0198] Protein in the filtered supernatant was stable for ≥30 days at -20°C and for ≥15 days at 2-8°C.

[0199] Protein was further purified on a cationic exchange column (SPFF, Amersham Biosciences) with elution using 350mM NaCl + 50 mM acetate + 5 M urea pH 5.00. The majority of impurities were present in the flow-thru. A pre-elution washing using a lower NaCl concentration (180 mM) advantageously eliminated two contaminating *E.coli* proteins.

[0200] The eluted material was adjusted to pH 8 (using 200 mM TRIS/HCl + 5 M urea pH 9) and further purified on a Q Sepharose HP column (Amersham) with elution using 150 mM NaCl + 20 mM TRIS/HCl pH 8.00 in 5 M urea. Again, a pre-elution washing with reduced salt (90 mM) was useful for eliminating impurities.

[0201] The filtered eluted material from Q HP column was diluted 1:2 using PBS pH 7.00 (150 mM NaCl + 10 mM potassium phosphate, pH 7.00) and then diafiltered against 10 volumes of PBS pH 7.00 by tangential ultrafiltration. At the end of diafiltration the material was concentrated 1.6 times to about 1.2 mg/ml total proteins. Using a 30,000 Da cut-off membrane (Regenerated Cellulose membrane 50cm², Millipore PLCTK 30) it was possible to dialyze the material with a yield of about 90%.

936-ΔG741 hybrid protein

[0202] DNA encoding protein 936 from meningococcal serogroup B strain 2996 and protein 741 from meningococcal serogroup B strain MC58 were digested and ligated, together with a short linker sequence, to give a plasmid encoding amino acid sequence SEQ ID 8. The plasmid was transfected into *E.coli* and bacteria were grown to express the protein. The recombinant protein was not secreted, but remained soluble within the bacteria.

[0203] After adequate growth, bacteria were centrifuged to give a humid paste and treated as follows:

- Homogenisation by high pressure system in presence of 20mM sodium phosphate pH 7.00.
- Centrifugation and clarification by orthogonal filtration.
- Cationic column chromatography (SP Sepharose Fast Flow), with elution by 150mM NaCl in 20mM sodium phosphate pH 7.00.
- Anionic column chromatography (Q Sepharose XL) with flow-through harvesting.
- Hydrophobic column chromatography (Phenyl Sepharose 6 Fast Flow High Sub) with elution by 20mM sodium phosphate, pH 7.00.
- Diafiltration against PBS pH 7.4 with a 10Kd cut-off.
- Final sterile filtration and storing at -20°C

[0204] Protein in the final material was stable for at least 3 months both at -20°C and at 2-8°C.

NadA^{(NL)(C)} protein

[0205] DNA encoding NadA protein from meningococcal serogroup B strain 2996 was digested to remove the sequence encoding its C-terminus, to give a plasmid encoding amino acid sequence SEQ ID 1. The plasmid was transfected into *E.coli* and bacteria were grown to express the protein. The recombinant protein was secreted into the culture medium, and the leader peptide was absent in the secreted protein (SEQ ID 2). The supernatant was treated as follows:

- Concentration 7X and diafiltration against buffer 20mM TRIS/HCl pH7.6 by cross flow UF (Cut off 30Kd).
- Anionic column chromatography (Q Sepharose XL), with elution by 400mM NaCl in 20mM TRIS/HCl pH 7.6.
- Hydrophobic column chromatography step (Phenyl Sepharose 6 Fast Flow High Sub), with elution by 50mM NaCl in TRIS/HCl pH 7.6.
- Hydroxylapatite ceramic column chromatography (HA Macro. Prep) with elution by 200mM sodium phosphate pH 7.4.
- Diafiltration (cut off 30Kd) against PBS pH 7.4
- Final sterile filtration and storing at -20°C

[0206] Protein in the final material was stable for at least 6 months both at -20°C and at 2-8°C.

[0207] NadA protein is susceptible to degradation, and truncated forms of NadA may be detected by western blot or by mass spectrometry (e.g. by MALDI-TOF) indicating up to 10kDa MW loss. Degradation products can be separated from native NadA by gel filtration (e.g. using column TSK 300SWXL, precolumn TSKSWXL, TOSOHAAS). Such filtration gives three peaks: (i) a first peak with retention time 12.637 min and apparent MW 885.036 Da; (ii) retention time 13.871 min and apparent MW 530.388 Da; (iii) retention time 13.871 min and apparent MW 530.388 Da. Light scattering analysis of the three peaks reveals real MW values of (i) 208500 Da, (ii) 98460 Da, (iii) 78760 Da. Thus the first peak contains NadA aggregates, and the third peak contains degradation products.

[0208] As the predicted molecular weight of NadA^{(NL)(C)} is 34.113 Da, peak (ii) contains a trimeric protein, which is the desired antigen.

Antigenic combinations

[0209] Mice were immunised with a composition comprising the three proteins and an aluminium hydroxide adjuvant. For comparison purposes, the three proteins were also tested singly. Ten mice were used per group. The mixture was able to induce high bactericidal titres against various strains:

	Meningococcal strain (Serogroup)							
	2996 (B)	MC58 (B)	NGH38	394/98 (B)	H44/76 (B)	F6124 (A)	BZ133 (C)	C11 (C)
(1)	32000	16000	130000	16000	32000	8000	16000	8000
(2)	256	131000	128	16000	32000	8000	16000	<4
(3)	32000	8000	-	-	-	8000	-	32000
Mix	32000	32000	65000	16000	260000	65000	>65000	8000

¹ indicates that this strain contains no NadA gene Looking at individual mice, the triple mixture induced high and consistent bactericidal titres against the three serogroup B strains from which the individual antigens are derived:

#	1	2	3	4	5	6	7	8	9	10
2996	32768	16384	65536	32768	32768	65536	65536	32768	65536	8192
MC58	65536	32768	65536	65536	65536	8192	65536	32768	32768	65536
394/98	65536	4096	16384	4096	8192	4096	32768	16384	8192	16384

Combination and comparison with OMVs

[0210] In further experiments, the adjuvanted antigens (20µg of each antigen per dose) were administered in combination with 10µg OMVs prepared either from strain H44/76 (Norway) or strain 394/98 (New Zealand). Positive controls were the anti-capsular SEAM-3 mAb for serogroup B or CRM197-conjugated capsular saccharides for other strains. Results (bactericidal titres) are shown in Table 1. The mixture almost always gives better titres than simple OMVs and, furthermore, the addition of the mixture to OMVs almost always significantly enhances the efficacy of the OMVs. Moreover, in many cases the antigen mixture matches or exceeds the response seen with the positive control.

Hypervirulent lineage tests

[0211] The following antigens were tested against a variety of serogroup B strains from a variety of hypervirulent lineages:

1. (a) NadA^{(NL)(C)}
2. (b) ΔG287-953
3. (c) 936-ΔG741
4. (d) a mixture of (a), (b) and (c)
5. (e) OMVs prepared from strain H44/76 (Norway)
6. (f) OMVs prepared from strain 394/98 (New Zealand)

7. (g) A mixture of Δ G287 and (e)
8. (h) A mixture of (d) and (e)
9. (i) A mixture of (d) and (f)

SEAM-3 was used as a positive control.

[0212] Results were as follows, expressed as the percentage of strains in the indicated hypervirulent lineage where the serum bactericidal titre exceeded 1024:

	# strains	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	S-3
A4	4	50	50	0	100	25	25	25	100	100	+
ET-5	8	25	75	88	100	71	14	71	100	100	+
Lineage 3	13	0	75	15	93	8	85	8	92	93	+
ET-37	4	11	22	0	33	0	0	0	22	25	+

[0213] Against particular reference strains, bactericidal titres were as follows:

	Strain	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	S-3
A4	961-5945	128	2048	<8	2048	262144	8192	262144	262144	4096	8192
ET-5	44/76	<4	2048	32768	131072	524288	8192	524288	524288	524288	16384
Lineage 3	394/98	<4	1024	32	4096	<4	16384	256	16384	16384	16384
ET-37	LPN17592	2048	1024	256	4096	<8	<8	512	16384	65536	1024

[0214] Compositions (d), (h) and (i) therefore induce bactericidal antibody responses against a wide variety of strains of serogroup B meningococcus from within hypervirulent lineages A4, ET-5 and lineage 3. Titres using compositions (h) and (i) were generally higher than with (d), but the coverage of strains within hypervirulent lineages A4, ET-5 and lineage 3 were no better.

[0215] Coverage of untyped strains was also high with compositions (d), (h) and (i).

Analysis of NadA N-terminus domain

[0216] Purified *N.meningitidis* NadA protein is known to bind to human epithelial cells [17] (e.g. Chang cells, HeLa cells, Hep-2 cells), and recombinant *E.coli* which express NadA display an adherent phenotype [18]. These *E.coli* are also able to invade epithelial cells, and intracellular NadA⁺ *E.coli* can be detected in Chang cells by immunofluorescence (after membrane permeabilisation) and by electron microscopy. NadA is thus believed function as an adhesin and an invasin for epithelial cells.

[0217] On the basis of secondary structure analysis, mature NadA has been subdivided into three putative domains: a N-terminal globular domain (aa 24-87), an α -helix internal region (aa 88-350) with high coiled-coil propensity, and a C-terminal membrane anchor (aa 351-405). The role of the N-terminal globular domain in host-cell interaction was investigated.

[0218] A truncated *nadA* gene coding for a protein devoid of amino acids 30-87 was cloned into pET-21 vector (pET-NadA Δ 30-87) and expressed in *E.coli* BL21(DE3) strain. Amino acids 24-29 were retained to allow processing of the leader peptide and correct maturation of the protein. Western blot and FACS analysis confirmed that NadA Δ 30-87 was expressed and formed oligomers on the *E.coli* cell surface i.e. deletion of the N-terminal domain does not interfere with the expression, export and membrane localization of NadA. However, the recombinant *E.coli* strain completely lost the capacity to adhere to Chang epithelial cells. The N-terminus domain is thus implicated in adhesin activity.

[0219] To further investigate which part of the N-terminal domain is involved in the interaction, the region was additionally divided into three putative sub-domains: amino acids 24-42, containing a predicted α -helix region with hydrophobic residues; amino acids 43-70, the internal part without a predicted defined secondary structure; and amino acids 71-87 containing an other predicted α -helix structure. Three constructs, each encoding a protein deleted of a single sub-domain, were generated and then introduced into *E. coli* BL21(DE3), obtaining the following strains: BL21(DE3)/pET-NadA Δ 24-42, BL21(DE3)/pET-NadA Δ 43-70 and BL21(DE3)/pET-NadA Δ 71-87. Surface localisation of the oligomers was confirmed by western blot and FACS analysis, but

adhesion to Chang epithelial cells was no better than the control BL21(DE3)/pET *E.coli* strain. These results, confirmed also using immunofluorescence microscopy analysis, indicate that the entire globular N-terminal domain of NadA is important in the interaction with human cells.

Combination with meningococcal and/or Hib conjugates

[0220] The triple MenB composition is combined with a mixture of oligosaccharide conjugates for serogroups C, W135 and Y, to give a vaccine containing the following antigens:

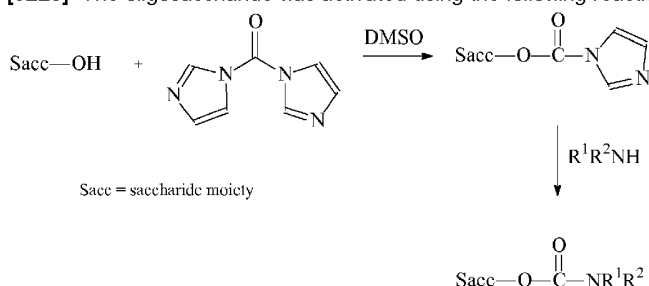
Component	Quantity per 0.5ml dose
Serogroup C conjugate	10 µg saccharide + 12.5-25 µg CRM ₁₉₇
Serogroup W135 conjugate	10 µg saccharide + 6.6-20 µg CRM ₁₉₇
Serogroup Y conjugate	10 µg saccharide + 6.6-20 µg CRM ₁₉₇
ΔG287-953	20 µg polypeptide
936-ΔG741	20 µg polypeptide
NadA	20 µg polypeptide

[0221] A similar vaccine is prepared, including MenA conjugate (10 µg saccharide + 12.5-33 µg CRM₁₉₇) and/or a HbOC Hib conjugate (10 µg saccharide + 2-5 µg CRM₁₉₇).

Use of modified MenA saccharide

[0222] Capsular polysaccharide was purified from MenA and was hydrolysed to give MenA oligosaccharide. The polysaccharide (2 g) was hydrolyzed at 50°C in 50 mM sodium acetate buffer, pH 4.75, at a polysaccharide concentration of 10 mg/mL for about 4 hours [135]. After hydrolysis, the solution was dried by rotary evaporation.

[0223] The oligosaccharide was activated using the following reaction scheme:



[0224] The oligosaccharide was dissolved in DMSO to give a saccharide concentration of 10 mg/mL. According to a molar ratio of oligosaccharide:CDI being 1:20, 21.262 g of CDI was then added and the reaction mixture stirred for 16 hours at room temperature. The resulting MenA-CDI compound was purified by selective precipitation in a 80:20 (v/v) acetone:DMSO mixture followed by centrifugation. The efficiency of the activation reaction was calculated to be about 67.9% by determining the ratio of free imidazole to bonded imidazole.

[0225] In the second reaction step, the MenA-CDI oligosaccharide was solubilised in DMSO at a saccharide concentration of about 10 mg/mL. According to a molar ratio of MenA-CDI unit:DMA being 1:100, 36.288 g of 99% dimethylamine hydrochloride (*i.e.* R¹ & R² = Me) was added and the reaction mixture stirred for 16 hours at room temperature. The reaction product was freeze-dried and resolubilised in 10 mg/mL water solution.

[0226] To remove the low molecular weight reaction reagent (in particular the dimethylamine (DMA)) from the oligosaccharide preparation, a dialysis step was performed through a 3.5 kDa MWCO membrane (Spectra/Por™). Four dialysis steps were carried out: (i) 16 hours against 2 L of 1 M sodium chloride (dialysis factor 1:20), (ii) 16 hours against 2 L of 0.5 M sodium chloride

(dialysis factor 1:20), (iii) and (iv) 16 hours against 2 L of WFI (dialysis factor 1:20). To improve the purification a diafiltration step was also performed through a 1 kDa MWCO membrane (Centricon™).

[0227] The purified MenA-CDI-DMA product was buffered at pH 6.5 in 25 mM L-histidine (Fluka™).

[0228] For preparing conjugates of the modified MenA saccharide (MenA-CDI-DMA), the overall process was as follows:

- hydrolysis of the polysaccharide to give oligosaccharide fragments
- sizing of the oligosaccharide fragments
- reductive amination of terminal aldehyde groups on the sized oligosaccharides
- protection of terminal -NH₂ groups by Fmoc group before the CDI reaction
- intrinsic de-protection of -NH₂ groups during the DMA reaction
- activation of terminal -NH₂ groups by SIDEA (N-hydroxysuccinimide adipic acid)
- covalent attachment to CRM₁₉₇ protein

[0229] The modified MenA oligosaccharide conjugate is much more resistant to hydrolysis than its natural counterpart at elevated temperatures. After 28 days at 37°C, for instance, the percentage of released saccharide is 6.4 % for the modified oligosaccharide vs. 23.5 % for the natural antigen. Moreover, the titres induced by the modified oligosaccharides are not significantly lower than those obtained using the native sugar structures.

[0230] The modified MenA conjugate is combined with MenC, MenW135 and MenY conjugates as a substitute for the conjugate of unmodified oligosaccharide. This tetravalent mixture is mixed with the three MenB polypeptides to give a vaccine effective against serogroups A, B, C, W135 and Y of *N.meningitidis* in a single dose.

Pneumococcal combinations

[0231] The three combined MenB proteins are mixed with pneumococcal saccharide conjugates to give a final concentration of 2µg/dose of each of the pneumococcal serotypes (double for serotype 6B). The reconstituted vaccine thus contains the following antigens:

Component	Quantity per 0.5ml dose
Serogroup A conjugate	5 µg saccharide + 6.25-16.5 µg CRM ₁₉₇
Serogroup C conjugate	5 µg saccharide + 6.25-12.5 µg CRM ₁₉₇
Serogroup W135 conjugate	5 µg saccharide + 3.3-10 µg CRM ₁₉₇
Serogroup Y conjugate	5 µg saccharide + 3.3-10 µg CRM ₁₉₇
Pneumococcus serotype 4 conjugate	2 µg saccharide + 2.5 µg CRM ₁₉₇
Pneumococcus serotype 9V conjugate	2 µg saccharide + 2.5 µg CRM ₁₉₇
Pneumococcus serotype 14 conjugate	2 µg saccharide + 2.5 µg CRM ₁₉₇
Pneumococcus serotype 18C conjugate	2 µg saccharide + 2.5 µg CRM ₁₉₇
Pneumococcus serotype 19F conjugate	2 µg saccharide + 2.5 µg CRM ₁₉₇
Pneumococcus serotype 23F conjugate	2 µg saccharide + 2.5 µg CRM ₁₉₇
Pneumococcus serotype 6B conjugate	4 µg saccharide + 5 µg CRM ₁₉₇

[0232] 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 of the claims.

REFERENCES

[0233]

- [1] Maiden et al. (1998) PNAS USA 95:3140-3145.
- [2] Armand et al. (1982) J. Biol. Stand. 10:335-339.
- [3] Cadoz et al. (1985) Vaccine 3:340-342.
- [4] Bjune et al. (1991) Lancet 338(8775):1093-96
- [5] Parkhill et al. (2000) Nature 404:502-506.
- [6] Tettelin et al. (2000) Science 287:1809-1815.
- [7] WO00/66791.
- [8] WO99/24578.
- [9] WO99/36544.
- [10] WO99/57280.
- [11] WO00/22430.
- [12] WO00/66741.
- [13] Pizza et al. (2000) Science 287:1816-1820.
- [14] WO01/64920.
- [15] WO01/64922.
- [16] WO03/020756.
- [17] Comanducci et al. (2002) J. Exp. Med. 195:1445-1454.
- [18] WO03/010194.
- [19] UK patent application 0227346.4.
- [20] WO03/063766.
- [21] Maignani et al. (2003) J Exp Med 197:789-799.
- [22] <http://neisseria.org/nml/typing/mlst/>
- [23] Pettersson et al. (1994) Microb Pathog 17(6):395-408.
- [24] Welsch et al. (2002) Thirteenth International Pathogenic Neisseria Conference, Norwegian Institute of Public Health, Oslo, Norway; Sept. 1-6, 2002. Genome-derived antigen (GNA) 2132 elicits protective serum antibodies to groups B and C *Neisseria meningitidis* strains.
- [25] Santos et al. (2002) Thirteenth International Pathogenic Neisseria Conference, Norwegian Institute of Public Health, Oslo, Norway; Sept. 1-6, 2002. Serum bactericidal responses in rhesus macaques immunized with novel vaccines containing recombinant proteins derived from the genome of *N. meningitidis*.
- [26] WO03/009869.
- [27] WO01/30390.
- [28] Almeida & Alpar (1996) J. Drug Targeting 3:455-467.
- [29] Agarwal & Mishra (1999) Indian J Exp Biol 37:6-16.
- [30] WO00/53221.
- [31] Jakobsen et al. (2002) Infect Immun 70:1443-1452.

- [32] Wu et al. (1997) *J Infect Dis* 175:839-846.
- [33] Bergquist et al. (1998) *APMIS* 106:800-806.
- [34] Baudner et al. (2002) *Infect Immun* 70:4785-4790.
- [35] Ugozzoli et al. (2002) *J Infect Dis* 186:1358-1361.
- [36] Paoletti et al. (2001) *Vaccine* 19:2118-2126.
- [37] WO00/56365.
- [38] Gennaro (2000) *Remington: The Science and Practise of Pharmacy*. 20th edition, ISBN: 0683306472.
- [39] *Vaccine Design...* (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.
- [40] WO00/23105.
- [41] WO90/14837.
- [42] US patent 5,057,540.
- [43] WO96/33739.
- [44] EP-A-0109942.
- [45] WO96/11711.
- [46] WO00/07621.
- [47] Barr et al. (1998) *Advanced Drug Delivery Reviews* 32:247-271.
- [48] Sjolander et al. (1998) *Advanced Drug Delivery Reviews* 32:321-338.
- [49] Niikura et al. (2002) *Virology* 293:273-280.
- [50] Lenz et al. (2001) *J Immunol* 166:5346-5355.
- [51] Pinto et al. (2003) *J Infect Dis* 188:327-338.
- [52] Gerber et al. (2001) *Virology* 75:4752-4760.
- [53] WO03/024480
- [54] WO03/024481
- [55] Gluck et al. (2002) *Vaccine* 20:B10-B16.
- [56] EP-A-0689454.
- [57] Johnson et al. (1999) *Bioorg Med Chem Lett* 9:2273-2278.
- [58] Evans et al. (2003) *Expert Rev Vaccines* 2:219-229.
- [59] Meraldi et al. (2003) *Vaccine* 21:2485-2491.
- [60] Pajak et al. (2003) *Vaccine* 21:836-842.
- [61] Kandimalla et al. (2003) *Nucleic Acids Research* 31:2393-2400.
- [62] WO02/26757.
- [63] WO99/62923.
- [64] Krieg (2003) *Nature Medicine* 9:831-835.
- [65] McCluskie et al. (2002) *FEMS Immunology and Medical Microbiology* 32:179-185.
- [66] WO98/40100.

- [67] US patent 6,207,646.
- [68] US patent 6,239,116.
- [69] US patent 6,429,199.
- [70] Kandimalla et al. (2003) Biochemical Society Transactions 31 (part 3):654-658.
- [71] Blackwell et al. (2003) J Immunol 170:4061-4068.
- [72] Krieg (2002) Trends Immunol 23:64-65.
- [73] WO01/95935.
- [74] Kandimalla et al. (2003) BBRC 306:948-953.
- [75] Bhagat et al. (2003) BBRC 300:853-861.
- [76] WO03/035836.
- [77] WO95/17211.
- [78] WO98/42375.
- [79] Beignon et al. (2002) Infect Immun 70:3012-3019.
- [80] Pizza et al. (2001) Vaccine 19:2534-2541.
- [81] Pizza et al. (2000) Int J Med Microbiol 290:455-461.
- [82] Scharton-Kersten et al. (2000) Infect Immun 68:5306-5313.
- [83] Ryan et al. (1999) Infect Immun 67:6270-6280.
- [84] Partidos et al. (1999) Immunol Lett 67:209-216.
- [85] Peppoloni et al. (2003) Expert Rev Vaccines 2:285-293.
- [86] Pine et al. (2002) J Control Release 85:263-270.
- [87] Domenighini et al. (1995) Mol Microbiol 15:1165-1167.
- [88] WO99/40936.
- [89] WO99/44636.
- [90] Singh et al] (2001) J Cont Release 70:267-276.
- [91] WO99/27960.
- [92] US patent 6,090,406
- [93] US patent 5,916,588
- [94] EP-A-0626169.
- [95] WO99/52549.
- [96] WO01/21207.
- [97] WO01/21152.
- [98] Andrianov et al. (1998) Biomaterials 19:109-115.
- [99] Payne et al. (1998) Adv Drug Delivery Review 31:185-196.
- [100] Stanley (2002) Clin Exp Dermatol 27:571-577.
- [101] Jones (2003) Curr Opin Investig Drugs 4:214-218.

- [102] WO99/11241.
- [103] WO94/00153.
- [104] WO98/57659.
- [105] European patent applications 0835318, 0735898 and 0761231.
- [106] WO96/37222; US patent 6,333,036..
- [107] Costantino et al. (1992) *Vaccine* 10:691-698.
- [108] WO03/007985.
- [109] Bell (2000) *Pediatr Infect Dis J* 19:1187-1188.
- [110] Iwarson (1995) *APMIS* 103:321-326.
- [111] Gerlich et al. (1990) *Vaccine* 8 Suppl:S63-68 & 79-80.
- [112] *Vaccines* (1988) eds. Plotkin & Mortimer. ISBN 0-7216-1946-0.
- [113] Del Giudice et al. (1998) *Molecular Aspects of Medicine* 19:1-70.
- [114] Gustafsson et al. (1996) *N. Engl. J. Med.* 334:349-355.
- [115] Rappuoli et al. (1991) *TIBTECH*9:232-238.
- [116] WO01/52885.
- [117] Fukasawa et al. (1999) *Vaccine* 17:2951-2958.
- [118] Rosenqvist et al. (1998) *Dev. Biol. Stand.* 92:323-333.
- [119] Sutter et al. (2000) *Pediatr Clin North Am* 47:287-308.
- [120] Zimmerman & Spann (1999) *Am Fam Physician* 59:113-118, 125-126.
- [121] Robinson & Torres (1997) *Seminars in Immunology* 9:271-283.
- [122] Donnelly et al. (1997) *Annu Rev Immunol* 15:617-648.
- [123] Scott-Taylor & Dalglish (2000) *Expert Opin Investig Drugs* 9:471-480.
- [124] Apostolopoulos & Plebanski (2000) *Curr Opin Mol Ther* 2:441-447.
- [125] Ilan (1999) *Curr Opin Mol Ther* 1:116-120.
- [126] Dubensky et al. (2000) *Mol Med* 6:723-732.
- [127] Robinson & Pertmer (2000) *Adv Virus Res* 55:1-74.
- [128] Donnelly et al. (2000) *Am J Respir Crit Care Med* 162(4 Pt 2):S190-193.
- [129] Davis (1999) *Mt. Sinai J. Med.* 66:84-90.
- [130] Charalambous & Feavers (2001) *J Med Microbiol* 50:937-939.
- [131] Westerink (2001) *Int Rev Immunol* 20:251-261.
- [132] Grothaus et al. (2000) *Vaccine* 18:1253-1263.
- [133] Jones (2001) *Curr Opin Investig Drugs* 2:47-49.
- [134] Ravenscroft et al. (1999) *Vaccine* 17:2802-2816.
- [135] Costantino et al. (1999) *Vaccine* 17:1251-1263.
- [136] WO03/080678.

- [137] Nilsson & Svensson (1979) Carbohydrate Research 69: 292-296)
- [138] Frash (1990) p.123-145 of Advances in Biotechnological Processes vol. 13 (eds. Mizrahi & Van Wezel)
- [139] Inzana (1987) Infect. Immun. 55:1573-1579.
- [140] Kandil et al. (1997) Glycoconj J 14:13-17.
- [141] Berkin et al. (2002) Chemistry 8:4424-4433.
- [142] Lindberg (1999) Vaccine 17 Suppl 2:S28-36.
- [143] Buttery & Moxon (2000) JR Coll Physicians Lond 34:163-168.
- [144] Ahmad & Chapnick (1999) Infect Dis Clin North Am 13:113-33, vii.
- [145] Goldblatt (1998) J. Med. Microbiol. 47:563-567.
- [146] European patent 0477508.
- [147] US patent 5,306,492.
- [148] WO98/42721.
- [149] Dick et al. in Conjugate Vaccines (eds. Cruse et al.) Karger, Basel, 1989, 10:48-114.
- [150] Hermanson Bioconjugate Techniques, Academic Press, San Diego (1996) ISBN: 0123423368.
- [151] Kanra et al. (1999) The Turkish Journal of Paediatrics 42:421-427.
- [152] Ravenscroft et al. (2000) Dev Biol (Basel) 103: 35-47.
- [153] WO97/00697.
- [154] WO02/00249.
- [155] Watson (2000) Pediatr Infect Dis J 19:331-332.
- [156] Rubin (2000) Pediatr Clin North Am 47:269-285, v.
- [157] Jedrzejewski (2001) Microbiol Mol Biol Rev 65:187-207.
- [158] Zielen et al. (2000) Infect. Immun. 68:1435-1440.
- [159] Darkes & Plosker (2002) Paediatr Drugs 4:609-630.
- [160] Tettelin et al. (2001) Science 293:498-506.
- [161] Hoskins et al (2001) J Bacteriol 183:5709-5717.
- [162] Rappuoli (2000) Curr Opin Microbiol 3:445-450
- [163] Rappuoli (2001) Vaccine 19:2688-2691.
- [164] Masignani et al. (2002) Expert Opin Biol Ther 2:895-905.
- [165] Mora et al. (2003) Drug Discov Today 8:459-464.
- [166] Witzemann et al. (2001) Infect Immun 69:1593-1598.
- [167] Rigden et al. (2003) Crit Rev Biochem Mol Biol 38:143-168.
- [168] WO02/22167.
- [169] Ramsay et al. (2001) Lancet 357(9251):195-196.
- [170] Anonymous (Jan 2002) Research Disclosure, 453077.
- [171] Anderson (1983) Infect Immun 39(1):233-238.

- [172] Anderson et al. (1985) J Clin Invest 76(1):52-59.
- [173] EP-A-0372501.
- [174] EP-A-0378881.
- [175] EP-A-0427347.
- [176] WO93/17712
- [177] WO94/03208.
- [178] WO98/58668.
- [179] EP-A-0471177.
- [180] WO91/01146
- [181] Falugi et al. (2001) Eur J Immunol 31:3816-3824.
- [182] EP-A-0594610.
- [183] WO00/56360.
- [184] WO02/091998.
- [185] WO01/72337
- [186] WO00/61761.
- [187] WO99/42130
- [188] WO96/40242
- [189] Lees et al. (1996) Vaccine 14:190-198.
- [190] WO95/08348.
- [191] US patent 4,882,317
- [192] US patent 4,695,624
- [193] Porro et al. (1985) Mol Immunol 22:907-919.s
- [194] EP-A-0208375
- [195] WO00/10599
- [196] Gever et al. Med. Microbiol. Immunol, 165 : 171-288 (1979).
- [197] US patent 4,057,685.
- [198] US patents 4,673,574; 4,761,283; 4,808,700.
- [199] US patent 4,459,286.
- [200] US patent 4,965,338
- [201] US patent 4,663,160.
- [202] US patent 4,761,283
- [203] US patent 4,356,170
- [204] Lei et al. (2000) Dev Biol (Basel) 103:259-264.
- [205] WO00/38711; US patent 6,146,902.
- [206] Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) Supplement 30.

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

TABLE I

	2996	NGH38	M4215	MC58	4476	CU365	N44/89	394/98	W15461/9	NW282	NW408	BZ198	W1545	G2136	5/99	F6124	BZ133	FW1750	265830
Typing:	B:9 P:36/3	B:81 P:13	B:15 P:11/8	B:15 P:11/6	B:15 P:11/6	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	B:4 P:14	A	C:WT	W:151	P:15
ET:	other	other	n.d.	ETS	ETS	ETS	ETS	lin.3	lin.3	lin.3	lin.3	lin.3	A4	A4	A4	slII	sl		
Positive control	32768	32768	32768	16384	16384	>16384	8192	16384	8192	32768	8192	16384	8192	32768	8192	1024		1024	4096
Antigen mixture	4096	4096	65536	32768	65536	>65536	>4096	8192	2048	>4096	4096	4096	2048	2048	>4096	8192	16384	4096	>8192
Antigens + H4476 DMVs	16384	8192	>65536	32768	524288	>65536	>4096	16384	8192	>4096	>4096	>4096	>8192	2048	>4096	32768	32768	16384	>8192
Antigens + 394/98 DMV	8192	8192	>65536	32768	>65536	>65536	>4096	65536	>8192	>4096	>4096	>4096	2048	8192	>4096	65536	65536	65536	>8192
DMVs (Nonseq)	<4	1024	8192	2048	262144	256	<8	4096	<4	<8	<8	<4	>8192	<8	<8	1024	<4	<8	>4096
DMVs (NZ)	512	<4	128	2048	<4	<8	<8	32768	>8192	4096	1024	4096	<15	n.d.	<8	4096	1024	<8	>4096

SEQUENCE LISTING

[0234]

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<130> P055397EP

<140> EP-10 _____. _

<141> 2003-10-02

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<212> PRT

<213> *Neisseria meningitidis*

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20     25     30
Ala Ala Thr Val Ala Ile Ala Ala Tyr Asn Asn Gly Gln Glu Ile
35     40     45
Asn Gly Phe Lys Ala Gly Glu Thr Ile Tyr Asp Ile Asp Glu Asp Gly
50     55     60
Thr Ile Thr Lys Lys Asp Ala Thr Ala Ala Asp Val Glu Ala Asp Asp
65     70     75     80
Phe Lys Gly Leu Gly Leu Lys Lys Val Val Thr Asn Leu Thr Lys Thr
85     90     95
Val Asn Glu Asn Lys Gln Asn Val Asp Ala Lys Val Lys Ala Ala Glu
100    105    110
Ser Glu Ile Glu Lys Leu Thr Thr Lys Leu Ala Asp Thr Asp Ala Ala
115    120    125
Leu Ala Asp Thr Asp Ala Ala Leu Asp Ala Thr Thr Asn Ala Leu Asn
130    135    140
Lys Leu Gly Glu Asn Ile Thr Thr Phe Ala Glu Glu Thr Lys Thr Asn
145    150    155    160
Ile Val Lys Ile Asp Glu Lys Leu Glu Ala Val Ala Asp Thr Val Asp
165    170    175
Lys His Ala Glu Ala Phe Asn Asp Ile Ala Asp Ser Leu Asp Glu Thr
180    185    190
Asn Thr Lys Ala Asp Glu Ala Val Lys Thr Ala Asn Glu Ala Lys Gln
195    200    205
Thr Ala Glu Glu Thr Lys Gln Asn Val Asp Ala Lys Val Lys Ala Ala
210    215    220
Glu Thr Ala Ala Gly Lys Ala Glu Ala Ala Ala Gly Thr Ala Asn Thr
225    230    235    240
Ala Ala Asp Lys Ala Glu Ala Val Ala Ala Lys Val Thr Asp Ile Lys
245    250    255
Ala Asp Ile Ala Thr Asn Lys Asp Asn Ile Ala Lys Lys Ala Asn Ser
260    265    270
Ala Asp Val Tyr Thr Arg Glu Glu Ser Asp Ser Lys Phe Val Arg Ile
275    280    285
Asp Gly Leu Asn Ala Thr Thr Glu Lys Leu Asp Thr Arg Leu Ala Ser
290    295    300
Ala Glu Lys Ser Ile Ala Asp His Asp Thr Arg Leu Asn Gly Leu Asp
305    310    315    320
Lys Thr Val Ser Asp Leu Arg Lys Glu Thr Arg Gln Gly Leu Ala Glu
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Gln Ala Ala Leu Ser Gly Leu Phe Gln Pro Tyr Asn Val Gly
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<212> PRT

<213> *Neisseria meningitidis*

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20     25     30
Thr Ile Tyr Asp Ile Asp Glu Asp Gly Thr Ile Thr Lys Lys Asp Ala
35     40     45
Thr Ala Ala Asp Val Glu Ala Asp Asp Phe Lys Gly Leu Gly Leu Lys
50     55     60
Lys Val Val Thr Asn Leu Thr Lys Thr Val Asn Glu Asn Lys Gln Asn
65     70     75     80

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Val Asp Ala Lys Val Lys Ala Ala Glu Ser Glu Ile Glu Lys Leu Thr
 85 90 95
 Thr Lys Leu Ala Asp Thr Asp Ala Ala Leu Ala Asp Thr Asp Ala Ala
 100 105 110
 Leu Asp Ala Thr Thr Asn Ala Leu Asn Lys Leu Gly Glu Asn Ile Thr
 115 120 125
 Thr Phe Ala Glu Glu Thr Lys Thr Asn Ile Val Lys Ile Asp Glu Lys
 130 135 140
 Leu Glu Ala Val Ala Asp Thr Val Asp Lys His Ala Glu Ala Phe Asn
 145 150 155 160
 Asp Ile Ala Asp Ser Leu Asp Glu Thr Asn Thr Lys Ala Asp Glu Ala
 165 170 175
 Val Lys Thr Ala Asn Glu Ala Lys Gln Thr Ala Glu Glu Thr Lys Gln
 180 185 190
 Asn Val Asp Ala Lys Val Lys Ala Ala Glu Thr Ala Ala Gly Lys Ala
 195 200 205
 Glu Ala Ala Ala Gly Thr Ala Asn Thr Ala Ala Asp Lys Ala Glu Ala
 210 215 220
 Val Ala Ala Lys Val Thr Asp Ile Lys Ala Asp Ile Ala Thr Asn Lys
 225 230 235 240
 Asp Asn Ile Ala Lys Lys Ala Asn Ser Ala Asp Val Tyr Thr Arg Glu
 245 250 255
 Glu Ser Asp Ser Lys Phe Val Arg Ile Asp Gly Leu Asn Ala Thr Thr
 260 265 270
 Glu Lys Leu Asp Thr Arg Leu Ala Ser Ala Glu Lys Ser Ile Ala Asp
 275 280 285
 His Asp Thr Arg Leu Asn Gly Leu Asp Lys Thr Val Ser Asp Leu Arg
 290 295 300
 Lys Glu Thr Arg Gln Gly Leu Ala Glu Gln Ala Ala Leu Ser Gly Leu
 305 310 315 320
 Phe Gln Pro Tyr Asn Val Gly
 325

<210> 3

<211> 248

<212> PRT

<213> *Neisseria meningitidis*

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 Leu Asp His Lys Asp Lys Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser
 20 25 30
 Val Arg Lys Asn Glu Lys Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys
 35 40 45

Thr Tyr Gly Asn Gly Asp Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp
 50 55 60
 Lys Val Ser Arg Phe Asp Phe Ile Arg Gln Ile Glu Val Asp Gly Gln
 65 70 75 80
 Leu Ile Thr Leu Glu Ser Gly Glu Phe Gln Val Tyr Lys Gln Ser His
 85 90 95
 Ser Ala Leu Thr Ala Phe Gln Thr Glu Gln Ile Gln Asp Ser Glu His
 100 105 110
 Ser Gly Lys Met Val Ala Lys Arg Gln Phe Arg Ile Gly Asp Ile Ala
 115 120 125
 Gly Glu His Thr Ser Phe Asp Lys Leu Pro Glu Gly Gly Arg Ala Thr
 130 135 140
 Tyr Arg Gly Thr Ala Phe Gly Ser Asp Asp Ala Gly Gly Lys Leu Thr
 145 150 155 160
 Tyr Thr Ile Asp Phe Ala Ala Lys Gln Gly Asn Gly Lys Ile Glu His
 165 170 175
 Leu Lys Ser Pro Glu Leu Asn Val Asp Leu Ala Ala Ala Asp Ile Lys
 180 185 190
 Pro Asp Gly Lys Arg His Ala Val Ile Ser Gly Ser Val Leu Tyr Asn
 195 200 205
 Gln Ala Glu Lys Gly Ser Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala
 210 215 220
 Gln Glu Val Ala Gly Ser Ala Glu Val Lys Thr Val Asn Gly Ile Arg
 225 230 235 240
 His Ile Gly Leu Ala Ala Lys Gln
 245

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<211> 179

<212> PRT

<213> *Neisseria meningitidis*

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 20 25 30
 Arg Ile Glu Thr Thr Ala Arg Ser Tyr Leu Arg Gln Asn Asn Gln Thr
 35 40 45
 Lys Gly Tyr Thr Pro Gln Ile Ser Val Val Gly Tyr Asn Arg His Leu
 50 55 60
 Leu Leu Leu Gly Gln Val Ala Thr Glu Gly Glu Lys Gln Phe Val Gly
 65 70 75 80
 Gln Ile Ala Arg Ser Glu Gln Ala Ala Glu Gly Val Tyr Asn Tyr Ile
 85 90 95
 Thr Val Ala Ser Leu Pro Arg Thr Ala Gly Asp Ile Ala Gly Asp Thr
 100 105 110
 Trp Asn Thr Ser Lys Val Arg Ala Thr Leu Leu Gly Ile Ser Pro Ala
 115 120 125
 Thr Gln Ala Arg Val Lys Ile Val Thr Tyr Gly Asn Val Thr Tyr Val
 130 135 140
 Met Gly Ile Leu Thr Pro Glu Glu Gln Ala Gln Ile Thr Gln Lys Val
 145 150 155 160
 Ser Thr Thr Val Gly Val Gln Lys Val Ile Thr Leu Tyr Gln Asn Tyr
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<210> 5

<211> 168

<212> PRT

<213> *Neisseria meningitidis*

<400> 5

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20     25     30
Gly Ser Val Glu Phe Asp Gln Ala Lys Arg Asp Gly Lys Ile Asp Ile
35     40     45
Thr Ile Pro Ile Ala Asn Leu Gln Ser Gly Ser Gln His Phe Thr Asp
50     55     60
His Leu Lys Ser Ala Asp Ile Phe Asp Ala Ala Gln Tyr Pro Asp Ile
65     70     75     80
Arg Phe Val Ser Thr Lys Phe Asn Phe Asn Gly Lys Lys Leu Val Ser
85     90     95
Val Asp Gly Asn Leu Thr Met His Gly Lys Thr Ala Pro Val Lys Leu
100    105    110
Lys Ala Glu Lys Phe Asn Cys Tyr Gln Ser Pro Met Glu Lys Thr Glu
115    120    125
Val Cys Gly Gly Asp Phe Ser Thr Thr Ile Asp Arg Thr Lys Trp Gly
130    135    140
Met Asp Tyr Leu Val Asn Val Gly Met Thr Lys Ser Val Arg Ile Asp
145    150    155    160
Ile Gln Ile Glu Ala Ala Lys Gln
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<210> 6

<211> 464

<212> PRT

<213> *Neisseria meningitidis*

<400> 6

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Val Val Ser Glu Lys Glu Thr Glu Ala Lys Glu Asp Ala Pro Gln Ala
20      25      30
Gly Ser Gln Gly Gln Gly Ala Pro Ser Ala Gln Gly Ser Gln Asp Met
35      40      45
Ala Ala Val Ser Glu Glu Asn Thr Gly Asn Gly Gly Ala Val Thr Ala
50      55      60
Asp Asn Pro Lys Asn Glu Asp Glu Val Ala Gln Asn Asp Met Pro Gln
65      70      75      80
Asn Ala Ala Gly Thr Asp Ser Ser Thr Pro Asn His Thr Pro Asp Pro
85      90      95
Asn Met Leu Ala Gly Asn Met Glu Asn Gln Ala Thr Asp Ala Gly Glu
100     105     110
Ser Ser Gln Pro Ala Asn Gln Pro Asp Met Ala Asn Ala Ala Asp Gly
115     120     125
Met Gln Gly Asp Asp Pro Ser Ala Gly Gly Gln Asn Ala Gly Asn Thr
130     135     140
Ala Ala Gln Gly Ala Asn Gln Ala Gly Asn Asn Gln Ala Ala Gly Ser
145     150     155     160
Ser Asp Pro Ile Pro Ala Ser Asn Pro Ala Pro Ala Asn Gly Gly Ser
165     170     175
Asn Phe Gly Arg Val Asp Leu Ala Asn Gly Val Leu Ile Asp Gly Pro
180     185     190
Ser Gln Asn Ile Thr Leu Thr His Cys Lys Gly Asp Ser Cys Ser Gly
195     200     205
Asn Asn Phe Leu Asp Glu Glu Val Gln Leu Lys Ser Glu Phe Glu Lys
210     215     220
Leu Ser Asp Ala Asp Lys Ile Ser Asn Tyr Lys Lys Asp Gly Lys Asn
225     230     235     240
Asp Lys Phe Val Gly Leu Val Ala Asp Ser Val Gln Met Lys Gly Ile
245     250     255
Asn Gln Tyr Ile Ile Phe Tyr Lys Pro Lys Pro Thr Ser Phe Ala Arg
260     265     270
Phe Arg Arg Ser Ala Arg Ser Arg Arg Ser Leu Pro Ala Glu Met Pro
275     280     285
Leu Ile Pro Val Asn Gln Ala Asp Thr Leu Ile Val Asp Gly Glu Ala
290     295     300
Val Ser Leu Thr Gly His Ser Gly Asn Ile Phe Ala Pro Glu Gly Asn
305     310     315     320
Tyr Arg Tyr Leu Thr Tyr Gly Ala Glu Lys Leu Pro Gly Gly Ser Tyr
325     330     335
Ala Leu Arg Val Gln Gly Glu Pro Ala Lys Gly Glu Met Leu Ala Gly
340     345     350
Ala Ala Val Tyr Asn Gly Glu Val Leu His Phe His Thr Glu Asn Gly
355     360     365
Arg Pro Tyr Pro Thr Arg Gly Arg Phe Ala Ala Lys Val Asp Phe Gly
370     375     380
Ser Lys Ser Val Asp Gly Ile Ile Asp Ser Gly Asp Asp Leu His Met
385     390     395     400
Gly Thr Gln Lys Phe Lys Ala Ala Ile Asp Gly Asn Gly Phe Lys Gly
405     410     415
Thr Trp Thr Glu Asn Gly Ser Gly Asp Val Ser Gly Lys Phe Tyr Gly
420     425     430
Pro Ala Gly Glu Glu Val Ala Gly Lys Tyr Ser Tyr Arg Pro Thr Asp
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<210> 7

<211> 644

<212> PRT

<213> Neisseria meningitidis

<400> 7

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20     25     30

Gln Ala Gly Ser Gln Gly Gln Gly Ala Pro Ser Ala Gln Gly Gly Gln
35     40     45

Asp Met Ala Ala Val Ser Glu Glu Asn Thr Gly Asn Gly Gly Ala Ala
50     55     60

Ala Thr Asp Lys Pro Lys Asn Glu Asp Glu Gly Ala Gln Asn Asp Met
65     70     75     80

Pro Gln Asn Ala Ala Asp Thr Asp Ser Leu Thr Pro Asn His Thr Pro
85     90     95

Ala Ser Asn Met Pro Ala Gly Asn Met Glu Asn Gln Ala Pro Asp Ala
100    105   110

Gly Glu Ser Glu Gln Pro Ala Asn Gln Pro Asp Met Ala Asn Thr Ala
115    120   125

Asp Gly Met Gln Gly Asp Asp Pro Ser Ala Gly Gly Glu Asn Ala Gly
130    135   140

Asn Thr Ala Ala Gln Gly Thr Asn Gln Ala Glu Asn Asn Gln Thr Ala
145    150   155   160

Gly Ser Gln Asn Pro Ala Ser Ser Thr Asn Pro Ser Ala Thr Asn Ser
165    170   175

Gly Gly Asp Phe Gly Arg Thr Asn Val Gly Asn Ser Val Val Ile Asp
180    185   190

Gly Pro Ser Gln Asn Ile Thr Leu Thr His Cys Lys Gly Asp Ser Cys
195    200   205

Ser Gly Asn Asn Phe Leu Asp Glu Glu Val Gln Leu Lys Ser Glu Phe
210    215   220

Glu Lys Leu Ser Asp Ala Asp Lys Ile Ser Asn Tyr Lys Lys Asp Gly
225    230   235   240

Lys Asn Asp Gly Lys Asn Asp Lys Phe Val Gly Leu Val Ala Asp Ser
245    250   255

Val Gln Met Lys Gly Ile Asn Gln Tyr Ile Ile Phe Tyr Lys Pro Lys
260    265   270

Pro Thr Ser Phe Ala Arg Phe Arg Arg Ser Ala Arg Ser Arg Arg Ser
275    280   285

Leu Pro Ala Glu Met Pro Leu Ile Pro Val Asn Gln Ala Asp Thr Leu
290    295   300

Ile Val Asp Gly Glu Ala Val Ser Leu Thr Gly His Ser Gly Asn Ile
305    310   315   320

Phe Ala Pro Glu Gly Asn Tyr Arg Tyr Leu Thr Tyr Gly Ala Glu Lys
325    330   335

Leu Pro Gly Gly Ser Tyr Ala Leu Arg Val Gln Gly Glu Pro Ser Lys
340    345   350

Gly Glu Met Leu Ala Gly Thr Ala Val Tyr Asn Gly Glu Val Leu His
355    360   365

Phe His Thr Glu Asn Gly Arg Pro Ser Pro Ser Arg Gly Arg Phe Ala
370    375   380

Ala Lys Val Asp Phe Gly Ser Lys Ser Val Asp Gly Ile Ile Asp Ser
385    390   395   400

Gly Asp Gly Leu His Met Gly Thr Gln Lys Phe Lys Ala Ala Ile Asp
405    410   415

Gly Asn Gly Phe Lys Gly Thr Trp Thr Glu Asn Gly Gly Gly Asp Val
420    425   430

Ser Gly Lys Phe Tyr Gly Pro Ala Gly Glu Glu Val Ala Gly Lys Tyr
435    440   445

Ser Tyr Arg Pro Thr Asp Ala Glu Lys Gly Gly Phe Gly Val Phe Ala
450    455   460

Gly Lys Lys Glu Gln Asp Gly Ser Gly Gly Gly Gly Ala Thr Tyr Lys
465    470   475   480

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Val Asp Glu Tyr His Ala Asn Ala Arg Phe Ala Ile Asp His Phe Asn
485 490 495

Thr Ser Thr Asn Val Gly Gly Phe Tyr Gly Leu Thr Gly Ser Val Glu
500 505 510

Phe Asp Gln Ala Lys Arg Asp Gly Lys Ile Asp Ile Thr Ile Pro Val
515 520 525

Ala Asn Leu Gln Ser Gly Ser Gln His Phe Thr Asp His Leu Lys Ser
530 535 540

Ala Asp Ile Phe Asp Ala Ala Gln Tyr Pro Asp Ile Arg Phe Val Ser
545 550 555 560

Thr Lys Phe Asn Phe Asn Gly Lys Lys Leu Val Ser Val Asp Gly Asn
565 570 575

Leu Thr Met His Gly Lys Thr Ala Pro Val Lys Leu Lys Ala Glu Lys
580 585 590

Phe Asn Cys Tyr Gln Ser Pro Met Ala Lys Thr Glu Val Cys Gly Gly
595 600 605

Asp Phe Ser Thr Thr Ile Asp Arg Thr Lys Trp Gly Val Asp Tyr Leu
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Ala Ala Lys Gln

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<211> 434

<212> PRT

<213> *Neisseria meningitidis*

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Leu Arg Ile Glu Thr Thr Ala Arg Ser Tyr Leu Arg Gln Asn Asn Gln
35 40 45

Thr Lys Gly Tyr Thr Pro Gln Ile Ser Val Val Gly Tyr Asn Arg His
50 55 60

Leu Leu Leu Leu Gly Gln Val Ala Thr Glu Gly Glu Lys Gln Phe Val
65 70 75 80

Gly Gln Ile Ala Arg Ser Glu Gln Ala Ala Glu Gly Val Tyr Asn Tyr
85 90 95

Ile Thr Val Ala Ser Leu Pro Arg Thr Ala Gly Asp Ile Ala Gly Asp
100 105 110

Thr Trp Asn Thr Ser Lys Val Arg Ala Thr Leu Leu Gly Ile Ser Pro
115 120 125

Ala Thr Gln Ala Arg Val Lys Ile Val Thr Tyr Gly Asn Val Thr Tyr
 130 135 140
 Val Met Gly Ile Leu Thr Pro Glu Glu Gln Ala Gln Ile Thr Gln Lys
 145 150 155 160
 Val Ser Thr Thr Val Gly Val Gln Lys Val Ile Thr Leu Tyr Gln Asn
 165 170 175
 Tyr Val Gln Arg Gly Ser Gly Gly Gly Gly Val Ala Ala Asp Ile Gly
 180 185 190
 Ala Gly Leu Ala Asp Ala Leu Thr Ala Pro Leu Asp His Lys Asp Lys
 195 200 205
 Gly Leu Gln Ser Leu Thr Leu Asp Gln Ser Val Arg Lys Asn Glu Lys
 210 215 220
 Leu Lys Leu Ala Ala Gln Gly Ala Glu Lys Thr Tyr Gly Asn Gly Asp
 225 230 235 240
 Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp Lys Val Ser Arg Phe Asp
 245 250 255
 Phe Ile Arg Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Ser
 260 265 270
 Gly Glu Phe Gln Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe
 275 280 285
 Gln Thr Glu Gln Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala
 290 295 300
 Lys Arg Gln Phe Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe
 305 310 315 320
 Asp Lys Leu Pro Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe
 325 330 335
 Gly Ser Asp Asp Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala
 340 345 350
 Ala Lys Gln Gly Asn Gly Lys Ile Glu His Leu Lys Ser Pro Glu Leu
 355 360 365
 Asn Val Asp Leu Ala Ala Ala Asp Ile Lys Pro Asp Gly Lys Arg His
 370 375 380
 Ala Val Ile Ser Gly Ser Val Leu Tyr Asn Gln Ala Glu Lys Gly Ser
 385 390 395 400
 Tyr Ser Leu Gly Ile Phe Gly Gly Lys Ala Gln Glu Val Ala Gly Ser
 405 410 415
 Ala Glu Val Lys Thr Val Asn Gly Ile Arg His Ile Gly Leu Ala Ala
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Lys Gln

<210> 9

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker

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 1 5

<210> 10

<211> 255

<212> PRT

<213> *Neisseria meningitidis*

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 20 25 30
 Ser Leu Thr Leu Asp Gln Ser Val Arg Lys Asn Glu Lys Leu Lys Leu
 35 40 45
 Ala Ala Gln Gly Ala Glu Lys Thr Tyr Gly Asn Gly Asp Ser Leu Asn
 50 55 60
 Thr Gly Lys Leu Lys Asn Asp Lys Val Ser Arg Phe Asp Phe Ile Arg
 65 70 75 80
 Gln Ile Glu Val Asp Gly Gln Leu Ile Thr Leu Glu Ser Gly Glu Phe
 85 90 95
 Gln Val Tyr Lys Gln Ser His Ser Ala Leu Thr Ala Phe Gln Thr Glu
 100 105 110
 Gln Ile Gln Asp Ser Glu His Ser Gly Lys Met Val Ala Lys Arg Gln
 115 120 125
 Phe Arg Ile Gly Asp Ile Ala Gly Glu His Thr Ser Phe Asp Lys Leu
 130 135 140
 Pro Glu Gly Gly Arg Ala Thr Tyr Arg Gly Thr Ala Phe Gly Ser Asp
 145 150 155 160
 Asp Ala Gly Gly Lys Leu Thr Tyr Thr Ile Asp Phe Ala Ala Lys Gln
 165 170 175
 Gly Asn Gly Lys Ile Glu His Leu Lys Ser Pro Glu Leu Asn Val Asp
 180 185 190
 Leu Ala Ala Ala Asp Ile Lys Pro Asp Gly Lys Arg His Ala Val Ile
 195 200 205
 Ser Gly Ser Val Leu Tyr Asn Gln Ala Glu Lys Gly Ser Tyr Ser Leu
 210 215 220
 Gly Ile Phe Gly Gly Lys Ala Gln Glu Val Ala Gly Ser Ala Glu Val
 225 230 235 240
 Lys Thr Val Asn Gly Ile Arg His Ile Gly Leu Ala Ala Lys Gln
 245 250 255

<210> 11

<211> 254

<212> PRT

<213> *Neisseria meningitidis*

<400> 11

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<212> PRT

<213> *Neisseria meningitidis*

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 20 25 30
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 35 40 45
 Gly Thr Leu Thr Leu Ser Ala Gln Gly Ala Glu Lys Thr Phe Lys Ala
 50 55 60
 Gly Asp Lys Asp Asn Ser Leu Asn Thr Gly Lys Leu Lys Asn Asp Lys
 65 70 75 80
 Ile Ser Arg Phe Asp Phe Val Gln Lys Ile Glu Val Asp Gly Gln Thr
 85 90 95
 Ile Thr Leu Ala Ser Gly Glu Phe Gln Ile Tyr Lys Gln Asn His Ser
 100 105 110
 Ala Val Val Ala Leu Gln Ile Glu Lys Ile Asn Asn Pro Asp Lys Thr
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 Glu His Thr Ala Phe Asn Gln Leu Pro Gly Gly Lys Ala Glu Tyr His
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 Gly Lys Ala Phe Ser Ser Asp Asp Pro Asn Gly Arg Leu His Tyr Ser
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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- [WO0066791A \[0233\]](#)
- [WO9924578A \[0233\]](#)
- [WO9936544A \[0233\]](#)
- [WO9957280A \[0233\]](#)
- [WO0022430A \[0233\]](#)
- [WO0066741A \[0233\]](#)
- [WO0164920A \[0233\]](#)
- [WO0164922A \[0233\]](#)
- [WO03020756A \[0233\]](#)
- [WO03010194A \[0233\]](#)
- [GB0227346A \[0233\]](#)
- [WO03063766A \[0233\]](#)

- [WO03009869A \[0233\]](#)
- [WO0130390A \[0233\]](#)
- [WO0053221A \[0233\]](#)
- [WO0056365A \[0233\]](#)
- [WO0023105A \[0233\]](#)
- [WO9014837A \[0233\]](#)
- [US5057540A \[0233\]](#)
- [WO9633739A \[0233\]](#)
- [EP0109942A \[0233\]](#)
- [WO9611711A \[0233\]](#)
- [WO0007621A \[0233\]](#)
- [WO03024480A \[0233\]](#)
- [WO03024481A \[0233\]](#)
- [EP0689454A \[0233\]](#)
- [WO0226757A \[0233\]](#)
- [WO9962923A \[0233\]](#)
- [WO9840100A \[0233\]](#)
- [US6207646B \[0233\]](#)
- [US6239116B \[0233\]](#)
- [US6429199B \[0233\]](#)
- [WO0195835A \[0233\]](#)
- [WO03035836A \[0233\]](#)
- [WO9517211A \[0233\]](#)
- [WO9842375A \[0233\]](#)
- [WO9940936A \[0233\]](#)
- [WO9944636A \[0233\]](#)
- [WO9927960A \[0233\]](#)
- [US6090406A \[0233\]](#)
- [US5916588A \[0233\]](#)
- [EP0626169A \[0233\]](#)
- [WO9952549A \[0233\]](#)
- [WO0121207A \[0233\]](#)
- [WO0121152A \[0233\]](#)
- [WO9911241A \[0233\]](#)
- [WO9400153A \[0233\]](#)
- [WO9857659A \[0233\]](#)
- [EP0835318A \[0233\]](#)
- [EP0735898A \[0233\]](#)
- [EP0761231A \[0233\]](#)
- [WO9637222A \[0233\]](#)
- [US6333036B \[0233\]](#)
- [WO03007985A \[0233\]](#)
- [WO0152885A \[0233\]](#)
- [WO03080678A \[0233\]](#)
- [EP0477508A \[0233\]](#)
- [US5306492A \[0233\]](#)
- [WO9842721A \[0233\]](#)
- [WO9700697A \[0233\]](#)
- [WO0200249A \[0233\]](#)
- [WO0222167A \[0233\]](#)
- [EP0372501A \[0233\]](#)
- [EP0378881A \[0233\]](#)
- [EP0427347A \[0233\]](#)
- [WO9317712A \[0233\]](#)
- [WO9403208A \[0233\]](#)
- [WO9858668A \[0233\]](#)
- [EP0471177A \[0233\]](#)
- [WO9101146A \[0233\]](#)

- [EP0594610A \[0233\]](#)
- [WO0056360A \[0233\]](#)
- [WO02091998A \[0233\]](#)
- [WO0172337A \[0233\]](#)
- [WO0061761A \[0233\]](#)
- [WO9942130A \[0233\]](#)
- [WO9640242A \[0233\]](#)
- [WO9508348A \[0233\]](#)
- [US4862317A \[0233\]](#)
- [US4685624A \[0233\]](#)
- [EP0208375A \[0233\]](#)
- [WO0010599A \[0233\]](#)
- [US4957685A \[0233\]](#)
- [US4673574A \[0233\]](#)
- [US4761283A \[0233\] \[0233\]](#)
- [US4808700A \[0233\]](#)
- [US4459286A \[0233\]](#)
- [US4965336A \[0233\]](#)
- [US4663160A \[0233\]](#)
- [US4356170A \[0233\]](#)
- [WO0038711A \[0233\]](#)
- [US6146902A \[0233\]](#)
- [EP03758486A \[0234\]](#)
- [WO00304848A \[0234\]](#)
- [GB0309115A \[0234\]](#)
- [GB0305831A \[0234\]](#)
- [GB0223741A \[0234\]](#)

Non-patent literature cited in the description

- **MAIDEN et al.**PNAS USA, 1998, vol. 95, 3140-3145 [0233]
- **ARMAND et al.**J. Biol. Stand., 1982, vol. 10, 335-339 [0233]
- **CADOZ et al.**Vaccine, 1985, vol. 3, 340-342 [0233]
- **BJUNE et al.**Lancet, 1991, vol. 338, 87751093-96 [0233]
- **PARKHILL et al.**Nature, 2000, vol. 404, 502-506 [0233]
- **TETTELIN et al.**Science, 2000, vol. 287, 1809-1815 [0233]
- **PIZZA et al.**Science, 2000, vol. 287, 1816-1820 [0233]
- **COMANDUCCI et al.**J. Exp. Med., 2002, vol. 195, 1445-1454 [0233]
- **MASIGNANI et al.**J Exp Med, 2003, vol. 197, 789-799 [0233]
- **PETTERSSON et al.**Microb Pathog, 1994, vol. 17, 6395-408 [0233]
- Thirteenth International Pathogenic Neisseria Conference**WELSCH et al.**Genome-derived antigen (GNA) 2132 elicits protective serum antibodies to groups B and C Neisseria meningitidis strainsNorwegian Institute of Public Health20020901 [0233]
- Serum bactericidal responses in rhesus macaques immunized with novel vaccines containing recombinant proteins derived from the genome of **NSANTOS et al.**Thirteenth International Pathogenic Neisseria ConferenceNorwegian Institute of Public Health20020901 [0233]
- **ALMEIDAALPARJ.** Drug Targeting, 1996, vol. 3, 455-467 [0233]
- **AGARWALMISHRA**Indian J Exp Biol, 1999, vol. 37, 6-16 [0233]
- **JAKOBSEN et al.**Infect Immun, 2002, vol. 70, 1443-1452 [0233]
- **WU et al.**J Infect Dis, 1997, vol. 175, 839-846 [0233]
- **BERGQUIST et al.**APMIS, 1998, vol. 106, 800-806 [0233]
- **BAUDNER et al.**Infect Immun, 2002, vol. 70, 4785-4790 [0233]
- **UGOZZOLI et al.**J Infect Dis, 2002, vol. 186, 1358-1361 [0233]
- **PAOLETTI et al.**Vaccine, 2001, vol. 19, 2118-2126 [0233]

- **GENNARO**Remington: The Science and Practise of Pharmacy20000000 [0233]
- Vaccine Design...Plenum19950000 [0233]
- **BARR et al.**Advanced Drug Delivery Reviews, 1998, vol. 32, 247-271 [0233]
- **SJOLANDERET et al.**Advanced Drug Delivery Reviews, 1998, vol. 32, 321-338 [0233]
- **NIIKURA et al.**Virology, 2002, vol. 293, 273-280 [0233]
- **LENZ et al.**J Immunol, 2001, vol. 166, 5346-5355 [0233]
- **PINTO et al.**J Infect Dis, 2003, vol. 188, 327-338 [0233]
- **GERBER et al.**Virology, 2001, vol. 75, 4752-4760 [0233]
- **GLUCK et al.**Vaccine, 2002, vol. 20, B10-B16 [0233]
- **JOHNSON et al.**Bioorg Med Chem Lett, 1999, vol. 9, 2273-2278 [0233]
- **EVANS et al.**Expert Rev Vaccines, 2003, vol. 2, 219-229 [0233]
- **MERALDI et al.**Vaccine, 2003, vol. 21, 2485-2491 [0233]
- **PAJAK et al.**Vaccine, 2003, vol. 21, 836-842 [0233]
- **KANDIMALLA et al.**Nucleic Acids Research, 2003, vol. 31, 2393-2400 [0233]
- **KRIEG**Nature Medicine, 2003, vol. 9, 831-835 [0233]
- **MCCLUSKIE et al.**FEMS Immunology and Medical Microbiology, 2002, vol. 32, 179-185 [0233]
- **KANDIMALLA et al.**Biochemical Society Transactions, 2003, vol. 31, 654-658 [0233]
- **BLACKWELL et al.**J Immunol, 2003, vol. 170, 4061-4068 [0233]
- **KRIEG**Trends Immunol, 2002, vol. 23, 64-65 [0233]
- **KANDIMALLA et al.**BBRC, 2003, vol. 306, 948-953 [0233]
- **BHAGAT et al.**BBRC, 2003, vol. 300, 853-861 [0233]
- **BEIGNON et al.**Infect Immun, 2002, vol. 70, 3012-3019 [0233]
- **PIZZA et al.**Vaccine, 2001, vol. 19, 2534-2541 [0233]
- **PIZZA et al.**Int J Med Microbiol, 2000, vol. 290, 455-461 [0233]
- **SCHARTON-KERSTEN et al.**Infect Immun, 2000, vol. 68, 5306-5313 [0233]
- **RYAN et al.**Infect Immun, 1999, vol. 67, 6270-6280 [0233]
- **PARTIDOS et al.**Immunol Lett, 1999, vol. 67, 209-216 [0233]
- **PEPPOLONI et al.**Expert Rev Vaccines, 2003, vol. 2, 285-293 [0233]
- **PINE et al.**J Control Release, 2002, vol. 85, 263-270 [0233]
- **DOMENIGHINI et al.**Mol Microbiol, 1995, vol. 15, 1165-1167 [0233]
- **SINGH et al.**J Cont Release, 2001, vol. 70, 267-276 [0233]
- **ANDRIANOV et al.**Biomaterials, 1998, vol. 19, 109-115 [0233]
- **PAYNE et al.**Adv Drug Delivery Review, 1998, vol. 31, 185-196 [0233]
- **STANLEY**Clin Exp Dermatol, 2002, vol. 27, 571-577 [0233]
- **JONES**Curr Opin Investig Drugs, 2003, vol. 4, 214-218 [0233]
- **COSTANTINO et al.**Vaccine, 1992, vol. 10, 691-698 [0233]
- **BELL**Pediatr Infect Dis J, 2000, vol. 19, 1187-1188 [0233]
- **IWARSON**APMIS, 1995, vol. 103, 321-326 [0233]
- **GERLICH et al.**Vaccine, 1990, vol. 8, 63-6879-80 [0233]
- Vaccines19880000 [0233]
- **DEL GUIDICE et al.**Molecular Aspects of Medicine, 1998, vol. 19, 1-70 [0233]
- **GUSTAFSSON et al.**N. Engl. J. Med., 1996, vol. 334, 349-355 [0233]
- **RAPPUOLI et al.**TIBTECH, 1991, vol. 9, 232-238 [0233]
- **FUKASAWA et al.**Vaccine, 1999, vol. 17, 2951-2958 [0233]
- **ROSENQVIST et al.**Dev. Biol. Stand., 1998, vol. 92, 323-333 [0233]
- **SUTTER et al.**Pediatr Clin North Am, 2000, vol. 47, 287-308 [0233]
- **ZIMMERMANS**PANNAm Fam Physician, 1999, vol. 59, 113-118125-126 [0233]
- **ROBINSON**TORRESSEminars in Immunology, 1997, vol. 9, 271-283 [0233]
- **DONNELLY et al.**Annu Rev Immunol, 1997, vol. 15, 617-648 [0233]
- **SCOTT-TAYLOR**DALGLEISHExpert Opin Investig Drugs, 2000, vol. 9, 471-480 [0233]
- **APOSTOLOPOULOS**PLEBANSKICurr Opin Mol Ther, 2000, vol. 2, 441-447 [0233]
- **ILAN**Curr Opin Mol Ther, 1999, vol. 1, 116-120 [0233]
- **DUBENSKY et al.**Mol Med, 2000, vol. 6, 723-732 [0233]
- **ROBINSON**PERTMERAdv Virus Res, 2000, vol. 55, 1-74 [0233]
- **DONNELLY et al.**Am J Respir Crit Care Med, 2000, vol. 162, 4190-193 [0233]
- **DAVIS**Mt. Sinai J. Med., 1999, vol. 66, 84-90 [0233]
- **CHARALAMBOUS**FEAVERSJ Med Microbiol, 2001, vol. 50, 937-939 [0233]
- **WESTERINK**Int Rev Immunol, 2001, vol. 20, 251-261 [0233]

- GROTHAUS et al. Vaccine, 2000, vol. 18, 1253-1263 [0233]
- JONES Curr Opin Investig Drugs, 2001, vol. 2, 47-49 [0233]
- RAVENSCROFT et al. Vaccine, 1999, vol. 17, 2802-2816 [0233]
- COSTANTINO et al. Vaccine, 1999, vol. 17, 1251-1263 [0233]
- NILSSONSVENSSON Carbohydrate Research, 1979, vol. 69, 292-296 [0233]
- FRASH Advances in Biotechnological Processes 19900000 vol. 13, 123-145 [0233]
- INZANA Infect. Immun., 1987, vol. 55, 1573-1579 [0233]
- KANDIL et al. Glycoconj J, 1997, vol. 14, 13-17 [0233]
- BERKIN et al. Chemistry, 2002, vol. 8, 4424-4433 [0233]
- LINDBERG Vaccine, 1999, vol. 17, 228-36 [0233]
- BUTTERYMOXON JR Coll Physicians Lond, 2000, vol. 34, 163-168 [0233]
- AHMADCHAPNICK Infect Dis Clin North Am, 1999, vol. 13, 113-33 [0233]
- GOLDBLATT J. Med. Microbiol., 1998, vol. 47, 563-567 [0233]
- DICK et al. Conjugate Vaccines 19890000 vol. 10, 48-114 [0233]
- HERMANSON Bioconjugate Techniques Academic Press 19960000 [0233]
- KANRA et al. The Turkish Journal of Paediatrics, 1999, vol. 42, 421-427 [0233]
- RAVENSCROFT et al. Dev Biol (Basel), 2000, vol. 103, 35-47 [0233]
- WATSON Pediatr Infect Dis J, 2000, vol. 19, 331-332 [0233]
- RUBIN Pediatr Clin North Am, 2000, vol. 47, 269-285 [0233]
- JEDRZEJAS Microbiol Mol Biol Rev, 2001, vol. 65, 187-207 [0233]
- ZIELEN et al. Infect. Immun., 2000, vol. 68, 1435-1440 [0233]
- DARKESPLOSKER Paediatr Drugs, 2002, vol. 4, 609-630 [0233]
- TETTELIN et al. Science, 2001, vol. 293, 498-506 [0233]
- HOSKINS et al. J Bacteriol, 2001, vol. 183, 5709-5717 [0233]
- RAPPUOLI Curr Opin Microbiol, 2000, vol. 3, 445-450 [0233]
- RAPPUOLI Vaccine, 2001, vol. 19, 2688-2691 [0233]
- MASIGNANI et al. Expert Opin Biol Ther, 2002, vol. 2, 895-905 [0233]
- MORA et al. Drug Discov Today, 2003, vol. 8, 459-464 [0233]
- WIZEMANN et al. Infect Immun, 2001, vol. 69, 1593-1598 [0233]
- RIGDEN et al. Crit Rev Biochem Mol Biol, 2003, vol. 38, 143-168 [0233]
- RAMSAY et al. Lancet, 2001, vol. 357, 9251195-196 [0233]
- ANONYMOUS Research Disclosure, 2002, 453077- [0233]
- ANDERSON Infect Immun, 1983, vol. 39, 1233-238 [0233]
- ANDERSON et al. J Clin Invest, 1985, vol. 76, 152-59 [0233]
- FALUGI et al. Eur J Immunol, 2001, vol. 31, 3816-3824 [0233]
- LEES et al. Vaccine, 1996, vol. 14, 190-198 [0233]
- PORRO et al. Mol Immunol, 1985, vol. 22, 907-919 [0233]
- GEVER et al. Med. Microbiol. Immunol, 1979, vol. 165, 171-288 [0233]
- LEI et al. Dev Biol (Basel), 2000, vol. 103, 259-264 [0233]
- Current Protocols in Molecular Biology 1987000030- [0233]
- SMITHWATERMAN Adv. Appl. Math., 1981, vol. 2, 482-489 [0233]

P A T E N T K R A V

1. Sammensætning omfattende fem meningokok-antigener:

(1) et NadA-protein, som er et polypeptid bestående af aminosyresekvensen ifølge SEQ ID NO: 2;

5 (2) et 741-protein, som har $\geq 85\%$ identitet med SEQ ID NO: 3;

(3) et 936-protein, som har $\geq 85\%$ identitet med SEQ ID NO: 4;

(4) et 953-protein, som har $\geq 85\%$ identitet med SEQ ID NO: 5; og

(5) et 287-protein, som har $\geq 85\%$ identitet med SEQ ID NO: 6.

10 2. Sammensætning ifølge krav 1, hvor sammensætningen omfatter et polypeptid ifølge formel $\text{NH}_2\text{-A-}[\text{-X-L-}]_n\text{-B-COOH}$, hvor: X er en aminosyresekvens af én af de fem antigener (1) til (5); L er en eventuel linker-aminosyresekvens; A er en eventuel N-terminal-aminosyresekvens; B er en eventuel C-terminal-aminosyresekvens; og n er 2, 3, 4 eller 5.

15

3. Sammensætning ifølge krav 2, hvor: (a) n er 2, X1 er 936-proteinet ifølge krav 1 og X2 er 741-proteinet ifølge krav 1; og/eller (b) n er 2, X1 er 287-proteinet ifølge krav 1 og X2 er 953-proteinet ifølge krav 1.

20

4. Sammensætning ifølge et hvilket som helst af krav 1 til 3, omfattende (a) et protein omfattende SEQ ID NO: 7 og/eller (b) et protein omfattende SEQ ID NO: 8.

5. Sammensætning ifølge et hvilket som helst af krav 1 til 4 omfattende en ydre-membran-vesikel-(OMV)-sammensætning fra N.meningitidis serogruppe B.