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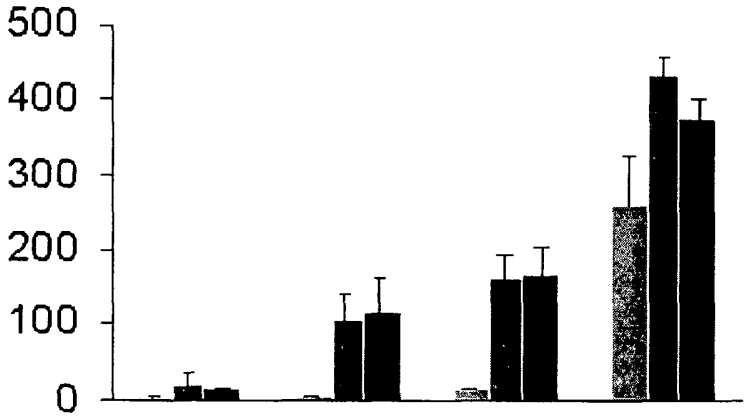

(57) Abstract: A composition comprising a Neisserial antigen and a detoxified ADP-ribosylating toxin. These compositions have been found to be useful for mucosal immunisation, particularly nasal immunisation against *Neisseria meningitidis*.

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ADJUVANTED ANTIGENIC MENINGOCOCCAL COMPOSITIONS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of vaccines, and in particular of vaccines against bacteria from the *Neisseria* genus (*e.g. N.gonorrhoeae* or, preferably, *N.meningitidis*).

BACKGROUND ART

References 1 to 6 disclose antigens, proteins and open reading frames from *Neisseria* bacteria, including *N.gonorrhoeae* and serogroups A and B of *N.meningitidis*. References 7 to 9 disclose various ways of expressing these proteins. Reference 10 discloses the enhancement of immunogenicity of *Neisseria* antigens when CpG adjuvants are used as adjuvants.

It is an object of the present invention to provide alternative and improved ways of enhancing immune responses raised against antigens from *Neisseria*, particularly *N.meningitidis* serogroup B.

DISCLOSURE OF THE INVENTION

The invention provides a composition comprising a Neisserial antigen and a detoxified ADP-ribosylating toxin. These compositions have been found to be useful for mucosal immunisation.

The composition is preferably an immunogenic composition, and more preferably a vaccine.

The Neisserial antigen

The Neisserial antigen is preferably a *N.meningitidis* antigen, and more preferably a *N.meningitidis* serogroup B antigen. Within serogroup B, preferred strains are 2996, MC58, 95N477, or 394/98.

The Neisserial antigen is preferably a protein antigen. More preferably, the protein antigen is selected from the group consisting of:

(a) a protein comprising one or more of the 446 even SEQ IDs (*i.e.* 2, 4, 6, ... , 890, 892) disclosed in reference 1.

(b) a protein comprising one or more of the 45 even SEQ IDs (i.e. 2, 4, 6, ... , 88, 90) disclosed in reference 2;

(c) a protein comprising one or more of the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241 disclosed in reference 3;

(d) a protein comprising one or more of the 2160 amino acid sequences NMB0001 to NMB2160 disclosed in reference 5;

(e) a protein comprising an amino acid sequence having sequence identity to an amino acid sequence specified in (a), (b), (c) or (d);

(f) a protein comprising a fragment of an amino acid sequence specified in (a), (b), (c) or (d);

(g) a protein comprising one or more of the amino acid sequences disclosed in reference 7, reference 8 or reference 9; or

(h) a protein having formula NH_2-A-[-X-L-]$_n$-B-COOH, wherein X is an amino acid sequence, 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 an integer greater than 1.

The degree of 'sequence identity' referred to in (e) is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more, up to 100%). This includes mutants, homologs, orthologs, allelic variants *etc.* [*e.g.* see reference 11]. Identity is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence.

The 'fragment' referred to in (f) should consist of least *m* consecutive amino acids from an amino acid sequence from (a), (b), (c), (d) or (e) and, depending on the particular sequence, *m* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more). Preferably the fragment comprises an epitope from an amino acid sequence from (a), (b), (c) or (d).

Preferred fragments are those disclosed in references 12 and 13. Other preferred fragments are C- and/or N-terminal truncations (*e.g.* Δ1-287, Δ2-287 *etc.*). Other preferred fragments omit poly-glycine sequences from the full-length sequence. This has been found to aid expression [ref. 8]. Poly-glycine sequences can be represented as $(Gly)_g$, where $g \geq 3$ (*e.g.* 4, 5, 6, 7, 8, 9 or more). If a -X- moiety in (h) includes a poly-glycine sequence in its wild-type form, it is preferred to omit this sequence in the hybrid proteins of the invention. This may be by disrupting or removing the $(Gly)_g$ – by deletion (*e.g.* CGGGGS→ CGGGS, CGGS, CGS or CS), by substitution (*e.g.* CGGGGS→ CGXGGS, CGXXGS, CGXGXS *etc.*), and/or by insertion (*e.g.* CGGGGS→ CGGXGGS, CGXGGGS, *etc.*). Deletion of $(Gly)_g$ is preferred, and this deletion is referred to herein as 'ΔG', particularly deletion of the whole N-terminus up to and including the $(Gly)_g$. Poly-glycine omission is particularly useful for proteins 287, 741, 983 and Tbp2 (ΔG287, ΔG741, ΔG983 and ΔGTbp2 [8]).

Other preferred fragments omit a leader peptide sequence from the full-length wild-type protein. This is particularly useful for proteins in group (h). In preferred proteins of group (h), all leader peptides in -X- moieties will be deleted except for that of the -X- moiety which is located at the N-terminus *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

Other preferred fragments omit complete protein domains. This is particularly useful for protein 961 ('NadA'), 287, and ORF46.1. Once a protein has been notional divided into domains, (c) and (j)

fragments can omit one or more of these domains (*e.g.* 287B, 287C, 287BC, $ORF46_{1-433}$, $ORF46_{433-608}$, ORF46, 961c – reference 8; Figures 8 and 9 in reference 9). 287 protein has been notionally split into three domains, referred to as A, B & C (see Figure 5 of reference 8). Domain B aligns strongly with IgA proteases, domain C aligns strongly with transferrin-binding proteins, and domain A shows no strong alignment with database sequences. An alignment of polymorphic forms of 287 is disclosed in reference 11. ORF46.1 has been notionally split into two domains – a first domain (amino acids 1-433) which is well-conserved between species and serogroups, and a second domain (amino acids 433-608) which is not well-conserved. The second domain is preferably deleted. An alignment of polymorphic forms of ORF46.1 is disclosed in reference 11. 961 protein has been notionally split into several domains (Figure 8 of reference 9).

Particularly preferred proteins in groups (a) to (d) comprise the amino acid sequence of (using the nomenclatures of references 1 to 9): orf1, orf4, orf25, orf40, orf46.1, orf83, NMB1343, 230, 233, 287, 292, 594, 687, 736, 741, 907, 919, 936, 953, 961 or 983. A preferred subset of these is: orf46.1, 230, 287, 741, 919, 936, 953, 961 and 983. A more preferred subset is: orf46.1, 287, 741 and 961.

In relation to group (h), the value of n is between 2 and x, and the value of x is typically 3, 4, 5, 6, 7, 8, 9 or 10. Preferably n is 2, 3 or 4; it is more preferably 2 or 3; most preferably, $n = 2$. Each -X- moiety is an amino acid sequence as specified in (a), (b), (c) or (d).

When n=2, preferred pairs of -X- moieties are: ΔG287 and 230; ΔG287 and 936; ΔG287 and 741; 961c and 287; 961c and 230; 961c and 936; 961cL and 287; 961cL and 230; 961cL and 936; ORF46.1 and 936; ORF46.1 and 230; 230 and 961; 230 and 741; 936 and 961; 936 and 741; ΔG741 and 741; ΔG287 and 287. Particularly preferred proteins are disclosed in references 14 and 15.

Where 287 is used in full-length form, it is preferably the final $-X_n-$ moiety; if it is to be used at the N-terminus (*i.e.* as $-X_1-$), it is preferred to use a ΔG form of 287.

For each n instances of [-X-L-], linker amino acid sequence -L- may be present or absent. For instance, when n=2 the hybrid may be $NH_2-X_1-L_1-X_2-L_2-COOH$, $NH_2-X_1-X_2-COOH$, $NH_2-X_1-L_1-X_2-COOH$, $NH_2-X_1-X_2-L_2-COOH$, *etc.*

Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* 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.

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.

-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.

-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.* 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.

The invention can use amino acid sequences from any strain of *N.meningitidis*. References to a particular protein (*e.g.* '287', or 'ORF46.1') therefore include that protein from any strain. Sequence variations between strains are included within (e) and (f).

Prototype sequences from *N.meningitidis* serogroup B include:

Protein	Prototype		Protein	Prototype
orf1	Ref. 1, SEQ ID 650		orf4	Ref. 1, SEQ ID 218
orf25	Ref. 1, SEQ ID 684		orf40	Ref. 2, SEQ ID 4
orf46.1	Ref. 8, Example 8		orf83	Ref. 1, SEQ ID 314
NMB1343	Ref. 5, NMB1343		230	Ref. 3, SEQ ID 830
233	Ref. 3, SEQ ID 860		287	Ref. 3, SEQ ID 3104
292	Ref. 3, SEQ ID 1220		594	Ref. 3, SEQ ID 1862
687	Ref. 3, SEQ ID 2282		736	Ref. 3, SEQ ID 2506
741	Ref. 3, SEQ ID 2536		907	Ref. 3, SEQ ID 2732
919	Ref. 3, SEQ ID 3070		936	Ref. 3, SEQ ID 2884
953	Ref. 3, SEQ ID 2918		961	Ref. 3, SEQ ID 940
983	Ref. 5, NMB1969			

Reference 11 discloses polymorphic forms of proteins ORF4, ORF40, ORF46, 225, 235, 287, 519, 726, 919 and 953. Polymorphic forms of 961 are disclosed in references 16 and 17. Reference 9 discloses polymorphic forms of 741, 961 and NMB1343. Reference 15 discloses polymorphic forms of 741. Any of these polymorphic forms may be used in accordance with the present invention.

Neisserial protein antigens are expressed in *Neisseria*, but the invention preferably utilises a heterologous host to express the antigen. 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, Salmonenna typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria (*e.g. M.tuberculosis*), yeast, *etc.*

The detoxified ADP-ribosylating toxin

ADP-ribosylating bacterial exotoxins are widely known. Examples include diphtheria toxin (*Corynebacterium diphtheriae*), exotoxin A (*Pseudomonas aeruginosa*), cholera toxin (CT; *Vibrio cholerae*), heat-labile enterotoxin (LT; *E.coli*) and pertussis toxin (PT).

The toxins catalyse the transfer of an ADP-ribose unit from NAD^+ to a target protein.

The toxins are typically divided into two functionally distinct domains – A and B. The A subunit is responsible for the toxic enzymatic activity, whereas the B subunit is responsible for cellular binding. The subunits might be domains on the same polypeptide chain, or might be separate polypeptide chains. The subunits may themselves be oligomers *e.g.* the A subunit of CT consists of A_1 and A_2 which are linked by a disulphide bond, and its B subunit is a homopentamer. Typically, initial contact with a target cell is mediated by the B subunit and then subunit A alone enters the cell.

The toxins are typically immunogenic, but their inclusion in vaccines is hampered by their toxicity. To remove toxicity without also removing immunogenicity, the toxins have been treated with chemicals such as glutaraldehyde or formaldehyde. A more rational approach relies on site-directed mutagenesis of key active site residues to remove toxic enzymatic activity whilst retaining immunogenicity [*e.g.* refs. 18 (CT and LT), 19 (PT), 20 *etc.*]. Current acellular whooping cough vaccines include a form of pertussis toxin with two amino acid substitutions ($Arg^9 \rightarrow Lys$ and $Glu^{129} \rightarrow Gly$; 'PT-9K/129G' [21]).

As well as their immunogenic properties, the toxins have been used as adjuvants. Parenteral adjuvanticity was first observed in 1972 [22] and mucosal adjuvanticity in 1984 [23]. It was surprisingly found in 1993 that the detoxified forms of the toxins retain adjuvanticity [24].

The compositions of the invention include a detoxified ADP-ribosylating toxin. The toxin may be diphtheria toxin, *Pseudomonas* exotoxin A or pertussis toxin, but is preferably cholera toxin (CT) or, more preferably, *E.coli* heat-labile enterotoxin (LT). Other toxins which can be used are those disclosed in reference 25 (SEQ IDs 1 to 7 therein).

Detoxification of these toxins without loss of immunogenic and/or adjuvant activity can be achieved by any suitable means, with mutagenesis being preferred. Mutagenesis may involve one or more substitutions, deletions and/or insertions.

Preferred detoxified mutants are LT having a mutation at residue Arg-7 (*e.g.* a Lys substitution); CT having a mutation at residue Arg-7 (*e.g.* a Lys substitution); CT having a mutation at residue Arg-11 (*e.g.* a Lys substitution); LT having a mutation at Val-53; CT having a mutation at Val-53; CT having a mutation at residue Ser-61 (*e.g.* a Phe substitution); LT having a mutation at residue Ser-63

(*e.g.* a Lys or Tyr substitution) [*e.g.* Chapter 5 of ref. 26 – K63]; CT having a mutation at residue Ser-63 (*e.g.* a Lys or Tyr substitution); LT having a mutation at residue Ala-72 (*e.g.* an Arg substitution) [27 – R72]; LT having a mutation at Val-97; CT having a mutation at Val-97; LT having a mutation at Tyr-104; CT having a mutation at Tyr-104; LT having a mutation at residue Pro-106 (*e.g.* a Ser substitution); CT having a mutation at residue Pro-106 (*e.g.* a Ser substitution); LT having a mutation at Glu-112 (*e.g.* a Lys substitution); CT having a mutation at Glu-112 (*e.g.* a Lys substitution); LT having a mutation at residue Arg-192 (*e.g.* a Gly substitution); PT having a mutation at residue Arg-9 (*e.g.* a Lys substitution); PT having a mutation at Glu-129 (*e.g.* a Gly substitution); and any of the mutants disclosed in reference 18.

These mutations may be combined *e.g.* Arg-9-Lys + Glu-129-Gly in PT.

LT with a mutation at residue 63 or 72 is a preferred detoxified toxin.

It will be appreciated that the numbering of these residues is based on prototype sequences and that, for example, although Ser-63 may not actually be the 63rd amino acid in a given LT variant, an alignment of amino acid sequences will reveal the location corresponding to Ser-63.

The detoxified toxins may be in the form of A and/or B subunits as appropriate for activity.

Mucosal administration

The composition of the invention is particularly suited to mucosal immunisation, although parenteral immunisation is also possible. Suitable routes of mucosal administration include oral, intranasal, intragastric, pulmonary, intestinal, rectal, ocular, and vaginal routes. Oral or intranasal administration is preferred.

The composition is preferably adapted for mucosal administration [*e.g.* see refs. 28, 29 & 30]. Where the composition is for oral administration, for instance, it may be in the form of tablets or capsules (optionally enteric-coated), liquid, transgenic plants *etc.* [see also ref. 31, and Chapter 17 of ref. 32].

Where the composition is for intranasal administration, it may be in the form of a nasal spray, nasal drops, gel or powder *etc* [*e.g.* ref. 33].

Further components of the composition

The composition of the invention will typically, in addition to the antigen and toxin 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, trehalose 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. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences*.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen, as well as any other of the above-mentioned 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. Dosage treatment may be a single dose schedule or a multiple dose schedule (*e.g.* including booster doses). The vaccine may be administered in conjunction with other immunoregulatory agents.

The composition may include other adjuvants in addition to detoxified toxin. Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO90/14837; Chapter 10 in ref. 32), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing MTP-PE) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) 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™); (2) saponin adjuvants, such as QS21 or Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent *e.g.* WO00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), *etc.*), interferons (*e.g.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) *e.g.* GB-2220221, EP-A-0689454; (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions *e.g.* EP-A-0835318, EP-A-0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs [Krieg *Vaccine* 2000, 19, 618-622; Krieg *Curr opin Mol Ther* 2001 3:15-24; Roman *et al.*, *Nat. Med.*, 1997, 3, 849-854; Weiner *et al.*, *PNAS USA*, 1997, 94, 10833-10837; Davis *et al.*, *J. Immunol.*, 1998, 160, 870-876; Chu *et al.*, *J. Exp. Med.*, 1997, 186, 1623-1631; Lipford *et al.*, *Eur. J. Immunol.*,

1997, 27, 2340-2344; Moldoveanu *et al., Vaccine*, 1988, 16, 1216-1224, Krieg *et al., Nature,* 1995, 374, 546-549; Klinman *et al., PNAS USA,* 1996, 93, 2879-2883; Ballas *et al., J. Immunol.,* 1996, 157, 1840-1845; Cowdery *et al., J. Immunol.,* 1996, 156, 4570-4575; Halpern *et al., Cell. Immunol.,* 1996, 167, 72-78; Yamamoto *et al., Jpn. J. Cancer Res.,* 1988, 79, 866-873; Stacey *et al., J. Immunol.,* 1996, 157, 2116-2122; Messina *et al., J. Immunol.,* 1991, 147, 1759-1764; Yi *et al., J. Immunol.,* 1996, 157, 4918-4925; Yi *et al., J. Immunol.,* 1996, 157, 5394-5402; Yi *et al., J. Immunol.,* 1998, 160, 4755-4761; and Yi *et al., J. Immunol.,* 1998, 160, 5898-5906; International patent applications WO96/02555, WO98/16247, WO98/18810, WO98/40100, WO98/55495, WO98/37919 and WO98/52581] *i.e.* containing at least one CG dinucleotide, with 5-methylcytosine optionally being used in place of cytosine; (8) a polyoxyethylene ether or a polyoxyethylene ester *e.g.* WO99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (*e.g.* WO01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (*e.g.* WO01/21152); (10) an immunostimulatory oligonucleotide (*e.g.* a CpG oligonucleotide) and a saponin *e.g.* WO00/62800; (11) an immunostimulant and a particle of metal salt *e.g.* WO00/23105; (12) a saponin and an oil-in-water emulsion *e.g.* WO99/11241; (13) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) *e.g.* WO98/57659; (14) PLG microparticles; (15) other substances that act as immunostimulating agents to enhance the efficacy of the composition.

Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

Compositions of the invention preferably comprise a buffer. Compositions of the invention are preferably buffered at between pH 6 and pH 8 (*e.g.* at about pH 7).

Compositions of the invention are preferably sterile and/or pyrogen-free.

Further antigens

Further antigens which can be included in the composition of the invention include:

- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 34, 35, 36, 37 *etc.*
- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 38 from serogroup C [see also ref. 39].
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* refs. 40, 41, 42].
- a protein antigen from *Helicobacter pylori* such as CagA [*e.g.* 43], VacA [*e.g.* 43], NAP [*e.g.* 44], HopX [*e.g.* 45], HopY [*e.g.* 45] and/or urease.
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 46, 47].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 47, 48].
- an antigen from hepatitis C virus [*e.g.* 49].

- 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. 50 & 51].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 52] *e.g.* the CRM_{197} mutant [*e.g.* 26].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 52].
- a saccharide antigen from *Haemophilus influenzae* B [*e.g.* 39].
- an antigen from *N.gonorrhoeae* [*e.g.* 1, 2, 3].
- an antigen from *Chlamydia pneumoniae* [*e.g.* 53, 54, 55, 56, 57, 58, 59].
- an antigen from *Chlamydia trachomatis* [*e.g.* 60].
- an antigen from *Porphyromonas gingivalis* [*e.g.* 61].
- polio antigen(s) [*e.g.* 62, 63] such as IPV or OPV.
- rabies antigen(s) [*e.g.* 64] such as lyophilised inactivated virus [*e.g.*65, RabAvert™].
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 52].
- influenza antigen(s) [*e.g.* chapter 19 of ref. 52], such as the haemagglutinin and/or neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [*e.g.* 66].
- an antigen from *Streptococcus agalactiae* (group B streptococcus) [*e.g.* 67, 68].
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 68, 69, 70].
- an antigen from *Staphylococcus aureus* [*e.g.* 71].

The composition may comprise one or more of these further antigens.

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity [*e.g.* refs. 72 to 81]. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [*e.g.* ref. 82], synthetic peptides [*e.g.* 83, 84], heat shock proteins [*e.g.* 85], pertussis proteins [*e.g.* 86, 87], protein D from *H.influenzae* [*e.g.* 88], toxin A or B from *C.difficile* [*e.g.* 89], *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it is preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Saccharides from different serogroups of *N.meningitidis* may be conjugated to the same or different carrier proteins.

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

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [51]).

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.

Antigens are preferably mixed with (and more preferably adsorbed to) an aluminium salt (*e.g.* phosphate, hydroxide, hydroxyphosphate, oxyhydroxide, orthophosphate, sulphate). The salt may take any suitable form (*e.g.* gel, crystalline, amorphous *etc.*).

Antigens in the composition 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.

As an alternative to using proteins antigens in the composition of the invention, nucleic acid encoding the antigen may be used [*e.g.* refs. 90 to 98]. 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. Such nucleic acid can be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*). The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

Medicaments

The composition of the invention is typically a vaccine composition.

The invention provides the compositions defined above for use as medicaments. The medicament is preferably able to raise an immune response in a mammal against the antigen (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention provides the use of the compositions defined above in the manufacture of a medicament for treating or preventing infection due to *Neisseria* bacteria (preferably *N.meningitidis*).

The invention provides a method of raising an immune response in an animal (*e.g.* a mammal, such as a mouse or a human), comprising administering to the animal a composition of the invention. The immune response is preferably protective. The animal is preferably 0-3 years old.

The invention provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition of the invention.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat disease after infection), but will typically be prophylactic.

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

The efficacy of an immunogenic composition can be assessed by monitoring antigen-specific immune responses (*e.g.* T cell or antibody responses) raised in an animal following administration of the composition.

The generation of bactericidal antibodies in an animal following administration of a composition of the invention is also indicative of efficacy.

Definitions

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.

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

BRIEF DESCRIPTION OF DRAWINGS

Figures 1 to 4 show IFN-γ (Figures 1 & 3; ng/ml) and IL-5 (Figures 2 and 4; pg/ml) levels induced by orf1 (Figures 1 & 2) and orf40 (figures 3 and 4). For figures 1 & 2, there are four data sets on the X-axis (left to right: PBS; orf1; orf1 + 1μg/ml LT-K63; orf1 + 1μg/ml LT-R72), each of which has three values for different antigen concentrations (left to right: 0.1μg/ml; 10μg/ml; 100μg/ml). For figures 3 & 4, there are five data sets on the X-axis (left to right: PBS; orf40; orf40 + 1μg/ml LT-K63; orf40 + 10μg/ml LT-K63; orf40 + 1μg/ml LT-R72), each of which has three values for different antigen concentrations (left to right: 0.1μg/ml; 10μg/ml; 100μg/ml).

Figure 5 shows IgG titres ($\log_{10}$) induced by orf1 and orf40. The seven columns are, from left to right: orf1; orf1 + 1μg/ml LT-K63; 1μg/ml LT-R72; orf40; orf40 + 1μg/ml LT-K63; orf40 + 10μg/ml LT-K63; orf40 + 1μg/ml LT-R72.

Figure 6 shows the IgG antibody subclass responses against ORF40. The four data sets are the same as the right-hand four sets in Figure 5. In each data set, the left-hand column shows IgG1 and the right-hand column shows IgG2a.

MODES FOR CARRYING OUT THE INVENTION

Intranasal immunisation with* N.meningitidis *protein antigens

Groups of five female Balb/c mice were immunised intranasally under ether anaesthesia on days 0, 21 and 42 with compositions comprising: (a) *N.meningitidis* (serogroup B) antigen orf1 or orf40; and (b) *E.coli* heat label toxin mutant R72 or K63. Negative control mice received either PBS or antigen without LT adjuvant. The groups were as follows:

Group	Antigen	Adjuvant
1	-	-
2	Orf1	-
3	Orf1	LT-K63 (1μg)
4	Orf1	LT-R72 (1μg)
5	Orf40	-
6	Orf40	LT-K63 (1μg)
7	Orf40	LT-K63 (10μg)
8	Orf40	LT-R72 (1μg)

Antigens (5μg/mouse) and adjuvants were delivered in a dose of 20μl per mouse.

Two weeks after the final immunisation, animals were sacrificed and blood, spleens and cervical lymph nodes were taken from mice. Sera was collected and stored for analysis of orf1/orf40-specific IgG, IgG1 and IgG2a by ELISA. Single cell suspensions were prepared from the spleens and cervical lymph nodes. The cells were set up in 96 well plates with orf1 or orf40 at 0, 0.1, 10 and 100μg/ml. As a positive control, the cells were incubated with PMA and anti-CD3. Cells were incubated at 37°C with 5% CO_2 for 3 days. On day 3, supernatants were collected for analysis of IL4, IL5 and interferon γ (IFNγ). ^{3}H-thymidine was added to the cells and plates were further incubated for 4 hr to allow an estimation of antigen-specific proliferation (data not shown).

Re-stimulated splenocytes from mice immunised with orf1 produced IFNγ and small amounts of IL-5 (figures 1 & 2). When mice were immunised with orf1 and LTK63 and in particular with LTR72 the cytokine responses were considerably stronger.

In contrast, immunisation with the antigen alone or together with the LT mutants did not lead to the production of significant levels of specific antibody (figure 5).

Immunisation with orf40 alone elicited a specific antibody response (figure 5). This was mainly of the IgG1 subclass (figure 6). Re-stimulated splenocytes from these mice did not produce significant amounts of IL-5 or IFNγ (figures 3 & 4). Immunisation with orf40 and LTK63 (1μg) resulted in a large increase in IL-5 production and increased IFNγ. Immunisation with orf40 and 10μg of LTK63 led to a higher IFNγ concentration in supernatants. Restimulated splenocytes from mice immunised with orf 40 and LTR72 (1μg) secreted IL-5 and IFNγ at concentrations comparable to those from mice immunised with the higher dose of LTK63. Sera from these mice contained high titres of specific IgG1 and IgG2a. In contrast, sera from mice immunised with orf40 and LTK63 contained higher titres of specific IgG1 than immunisation with the antigen alone. However, titres of specific IgG2a were not enhanced. Immunisation with the 10μg dose of LTK63 resulted in higher titres of specific IgG2a. This correlated with the higher production of IFNγ in this group. The individual titres

of orf40-specific IgG1 and IgG2a are presented in Table 1. These will be required to set up the assays for bactericidal antibody titres.

Table. 1. Individual IgG1 and IgG2a subclass titres from mice immunised with orf40 alone or together with LTK63/LTR72

orf40 only		orf40 + K63(1μg)		orf40+K63(10μg)		Orf40 + R72 (1μg)	
IgG1	IgG2a	IgG1	IgG2a	IgG1	IgG2a	IgG1	IgG2a
1	1	25000	1	100000	2500	15000	100
600	1	40000	1	100000	15000	15000	900
1500	1	60000	1	100000	35000	50000	9000
4000	1	80000	2500	150000	70000	150000	10000
30000	1000	100000	25000	500000	80000	800000	150000

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

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CLAIMS

1. A composition comprising a Neisserial antigen and a detoxified ADP-ribosylating toxin.

2. The composition of claim 1, wherein the Neisserial antigen is a *N.meningitidis* antigen.

3. The composition of claim 2, wherein the Neisserial antigen is a *N.meningitidis* serogroup B protein antigen.

4. The composition of claim 3, wherein the antigen is selected from the group consisting of:

   (a) a protein comprising one or more of the 446 even SEQ IDs (i.e. 2, 4, 6, ... , 890, 892) disclosed in WO99/24578.

   (b) a protein comprising one or more of the 45 even SEQ IDs (i.e. 2, 4, 6, ... , 88, 90) disclosed in reference WO99/36544;

   (c) a protein comprising one or more of the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241 disclosed in reference WO99/57280;

   (d) a protein comprising one or more of the 2160 amino acid sequences NMB0001 to NMB2160;

   (e) a protein comprising an amino acid sequence having at least 50% sequence identity to an amino acid sequence specified in (a), (b), (c) or (d);

   (f) a protein comprising a fragment of at least 7 amino acids of an amino acid sequence specified in (a), (b), (c) or (d);

   (g) a protein comprising one or more of the amino acid sequences disclosed in WO01/64920 or WO01/64922 or PCT/IB02/03904; or

   (h) a protein having formula NH_2-A-[-X-L-]$_n$-B-COOH, wherein X is an amino acid sequence, 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 an integer greater than 1.

5. The composition of claim 3 or claim 4, wherein the antigen comprises the amino acid sequence of orf1, orf4, orf25, orf40, orf46.1, orf83, NMB1343, 230, 233, 287, 292, 594, 687, 736, 741, 907, 919, 936, 953, NadA or 983.

6. The composition of claim 5, wherein the antigen comprises the amino acid sequence of orf46.1, 230, 287, 741, 919, 936, 953, NadA or 983.

7. The composition of claim 6, wherein the antigen comprises the amino acid sequence of orf46.1, 287, 741 or NadA.

8. The composition of claim 4, wherein the antigen is a protein having formula NH_2-A-[-X-L-]$_n$-B-COOH, wherein X is an amino acid sequence, 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 an integer greater than 1.

9. The composition of claim 8, wherein the value of *n* is between 2 and 10.

10. The composition of claim 8 or claim 9, wherein each -X- moiety is selected from the group consisting of: ΔG287; 230; 936; 741; 961c; 287; 961cL; ORF46.1; ΔG741.

11. The composition of any one of claims 8 to 10, wherein -L- has fewer than 20 amino acids.

12. The composition of any one of claims 8 to 11, wherein -A- has fewer than 40 amino acids and/or -B- has fewer than 40 amino acids.

13. The composition of any preceding claim, wherein the ADP-ribosylating toxin is cholera toxin or *E.coli* heat-labile enterotoxin.

14. The composition of claim 13, wherein the toxin is LT having a mutation at residue Ser-63 or Ala-72.

15. The composition of claim 14, wherein the toxin is LT-K63 or LT-R72.

16. The composition of any preceding claim, wherein the composition is adapted for mucosal administration.

17. The composition of claim 16, wherein the composition is adapted for intranasal administration.

18. The composition of any preceding claim, wherein the composition further comprises one or more of the following antigens:

- an outer-membrane vesicle (OMV) preparation from *N.meningitidis*;
- a saccharide antigen from *N.meningitidis*;
- a saccharide antigen from *Streptococcus pneumoniae*;
- an antigen from hepatitis A, B or C virus;
- an antigen from *Bordetella pertussis*;
- a diphtheria antigen;
- a tetanus antigen;
- a protein antigen from *Helicobacter pylori*;
- a saccharide antigen from *Haemophilus influenzae*;
- an antigen from *N.gonorrhoeae*;

– an antigen from *Chlamydia pneumoniae*;
– an antigen from *Chlamydia trachomatis*;
– an antigen from *Porphyromonas gingivalis*;
– polio antigen(s);
– rabies antigen(s);
– measles, mumps and/or rubella antigens;
– influenza antigen(s);
– an antigen from *Moraxella catarrhalis*;
– an antigen from *Streptococcus agalactiae*;
– an antigen from *Streptococcus pyogenes*; and/or
– an antigen from *Staphylococcus aureus*.

19. The composition of any preceding claim, further comprising a buffer.

20. The composition of any preceding claim, having a pH or between 6 and 8.

21. The composition of any preceding claim, wherein the composition is sterile and/or pyrogen-free.

22. The composition of any one of claims 1 to 21 for use as a medicament.

23. The use of the composition of any one of claims 1 to 21 in the manufacture of a medicament for treating or preventing infection due to *Neisseria* bacteria.

24. A method of raising an immune response in an animal, comprising administering to the animal a composition of any one of claims 1 to 21.

25. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition of any one of claims 1 to 21.

26. The composition of any one of claims 1 to 21, wherein the composition is an immunogenic composition.

27. The composition of any one of claims 1 to 21, wherein the composition is a vaccine.

28. The composition of any one of claims 1 to 21, wherein the Neisserial antigen is orf1 or orf40.

FIGURE 1

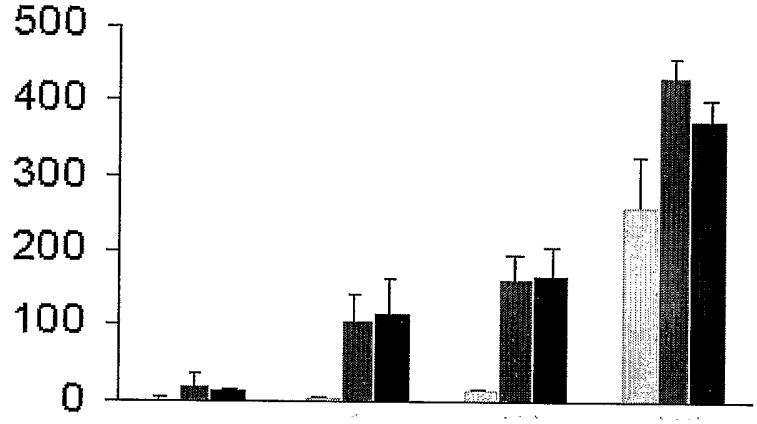

FIGURE 2

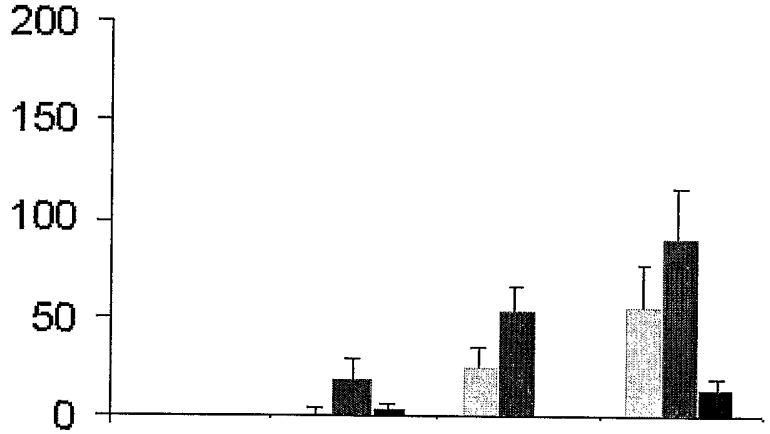

FIGURE 3

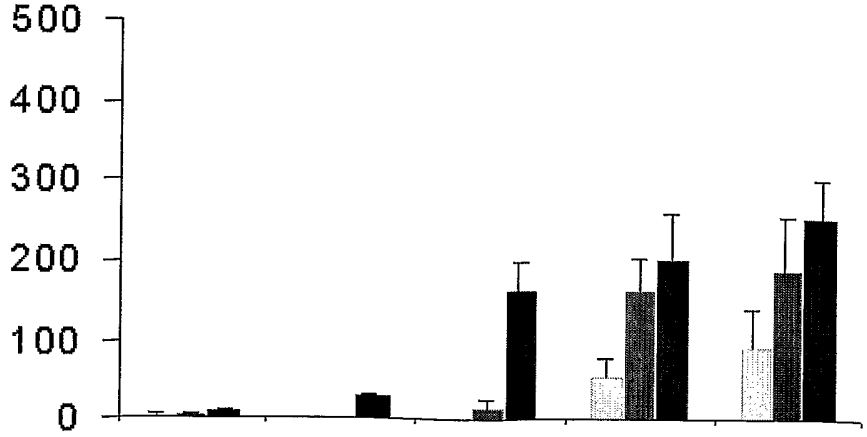

FIGURE 4

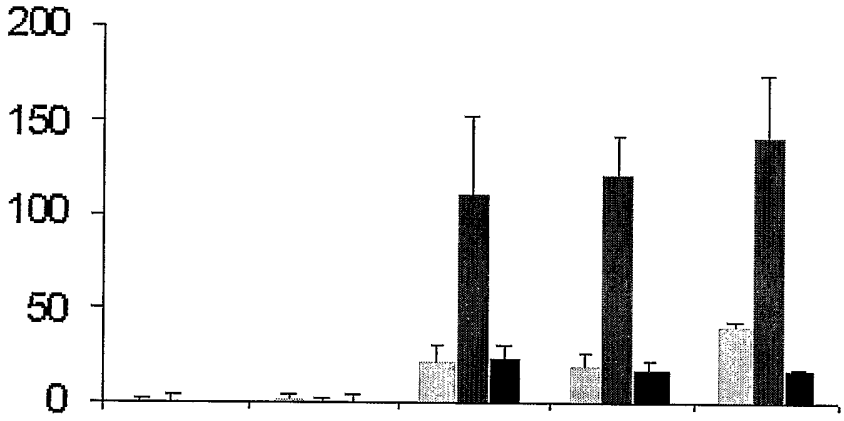

FIGURE 5

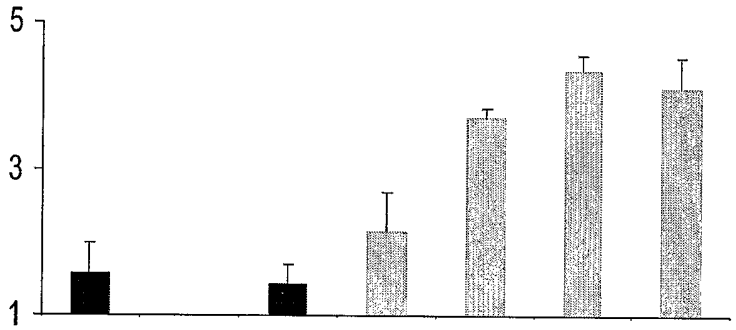

FIGURE 6