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- (71) Applicant(s)
Novartis Vaccines and Diagnostics SRL
- (72) Inventor(s)
Oster, Philipp;Pizza, Mariagrazia;Rappuoli, Rino
- (74) Agent / Attorney
FB Rice, Level 23 44 Market Street, Sydney, NSW, 2000
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(71) Applicant (for all designated States except US): **CHIRON SRL** [IT/IT]; Via Fiorentina, 1, I-53100 Siena (IT).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **OSTER, Philipp** [NL/IT]; Chiron Vaccines, Via Fiorentina, 1, I-53100 Siena (IT).

(74) Agents: **MARSHALL, Cameron, John** et al.; Carpmiels & Ransford, 43-45 Bloomsbury Square, London WC1A 2RA (GB).

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(54) Title: IMPROVEMENTS RELATING TO MENINGOCOCCAL OUTER MEMBRANE VESICLES

(57) Abstract: OMVs targeted against specific epidemic strains can be highly effective in controlling localised outbreaks of disease. In combination with large-scale and reproducible manufacturing techniques, a vaccine can be rapidly produced after an outbreak. The invention provides a method for preparing a meningococcal outer membrane vesicle (OMV) vaccine, comprising the steps of: (i) identifying the serosubtype of a meningococcal strain associated with an outbreak of meningococcal meningitis; (ii) preparing OMVs from a meningococcal strain having the serosubtype identified in step (i) for use in vaccine manufacture. The method may comprise one or both of the further steps of (iii) formulating said OMVs as a vaccine; and (iv) distributing said vaccine in a geographical area affected by or likely to be affected by said outbreak. The meningococcal strain will typically be in serogroup B, but may be instead by in serogroup A, C, W135, Y, etc.



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IMPROVEMENTS RELATING TO MENINGOCOCCAL OUTER MEMBRANE VESICLES

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

RELATED APPLICATIONS DATA

This application is an Australian national phase filing of International Patent Application No. PCT/IB2005/012801 (publication No. WO 2006/024946) filed on September 5, 2005, which claims priority from Great Britain Patent Application No. 0419627.5 filed on September 3, 2004, the contents of which are incorporated herein in their entirety by way of reference.

TECHNICAL FIELD

This invention is in the field of meningococcal outer membrane vesicles for immunisation purposes.

BACKGROUND ART

One of the various approaches to immunising against infection by *Neisseria meningitidis* (meningococcus) is to use outer membrane vesicles (OMVs). An efficacious OMV vaccine against serogroup B has been produced by the Norwegian National Institute of Public Health ['MenBvacTM'; e.g. ref. 1] but, although this vaccine is safe and prevents MenB disease, efficacy is limited to the homologous serotype used to make the vaccine.

The 'RIVM' vaccine is based on OMVs containing six different PorA subtypes. It has been shown to be immunogenic in children in phase II clinical trials [2].

Reference 3 discloses a vaccine against different pathogenic serotypes of serogroup B meningococcus based on OMVs which retain a protein complex of 65-kDa. Reference 4 discloses a vaccine comprising OMVs from genetically engineered meningococcal strains, with the OMVs comprising: at least one Class 1 outer-membrane protein (OMP) but not comprising a Class 2/3 OMP. Reference 5 discloses OMVs comprising OMPs which have mutations in their surface loops and OMVs comprising derivatives of meningococcal lipopolysaccharide (LPS). Reference 6 discloses a process for preparing OMV-based vaccines for serogroup A meningococcus.

There have been various proposals to improve OMV efficacy. Reference 7 discloses compositions comprising OMVs supplemented with transferrin binding proteins (e.g. TbpA and TbpB) and/or Cu,Zn-superoxide dismutase. Reference 8 discloses compositions comprising OMVs supplemented by various proteins. Reference 9 discloses preparations of membrane vesicles obtained from *N.meningitidis* with a modified *fur* gene. Reference 26 teaches that *nspA* expression should be up regulated with concomitant *porA* and *cps* knockout. Further knockout mutants of *N.meningitidis* for OMV production are disclosed in references 26 to 28. In contrast to these attempts to improve OMVs by changing expression patterns, reference 29 focuses on changing the methods for OMV preparation, and teaches that antigens such as NspA can be retained during vesicle extraction by avoiding the use of detergents such as deoxycholate.

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The failure of meningococcal OMVs to elicit cross-protection against non-homologous serotypes limits their use as general vaccines, but they can be very useful in epidemic situations where disease is characterised by pathogenic strains that are essentially clonal. Thus the Finlay Institute vaccine (VA-MENGOC-BC™) has been useful in Latin America, where serogroup B disease had been dominated by the P1.19,15 serotype, but has not been effective elsewhere [10]. Similarly, the Chiron MeNZB™ vaccine has been targeted at the epidemic strain (P1.7b,4, known as P1.7-2,4 by recent nomenclature) that has been prevalent in New Zealand since 1991.

Reference 11 discloses vaccine comprising multivalent meningococcal bleb compositions, comprising a first bleb derived from a meningococcal strain with a serosubtype prevalent in a country of use, and a second bleb derived from a strain that need not have a serosubtype prevalent in a country of use.

In one example the present invention provides further and improved meningococcal OMV preparations.

DISCLOSURE OF THE INVENTION

Experience with MeNZB™ has shown that OMVs targeted against specific epidemic strains can be highly effective in controlling localised outbreaks of disease. In combination with large-scale and reproducible manufacturing techniques, a vaccine can be rapidly produced after an outbreak. Thus the invention provides a method for preparing a meningococcal outer membrane vesicle (OMV) vaccine, comprising the steps of: (i) identifying the serosubtype of a meningococcal strain associated with an outbreak of meningococcal meningitis; (ii) preparing OMVs from a meningococcal strain having the serosubtype identified in step (i) for use in vaccine manufacture. The method may comprise one or both of the further steps of: (iii) formulating said OMVs as a vaccine; and (iv) distributing said vaccine in a geographical area affected by or likely to be affected by said outbreak. The invention also provides the same method, but omitting step (i), for situations where the relevant serosubtype has already been identified. The meningococcal strain will typically be in serogroup B, but may be instead by in serogroup A, C, W135, Y, *etc.*

Experience with MeNZB™ has also suggested to the inventors that OMVs will be useful for immunising against meningococcal serogroups A, C, W135 and Y, either alone or in combination, and that these might be cheaper to manufacture than the currently-proposed conjugate vaccines. Thus the invention provides: (a) a composition comprising outer membrane vesicles from a serogroup C strain of meningococcus; (b) a composition comprising outer membrane vesicles from a serogroup W135 strain of meningococcus; (c) a composition comprising outer membrane vesicles from a serogroup Y strain of meningococcus; and (d) a composition comprising outer membrane vesicles from two or more of serogroups A, B, C, W135 and Y of meningococcus. Within (d), preferred compositions include the following serogroup mixtures: A+B; A+C; A+W135; A+Y; B+C; B+W135; B+Y; C+W135; C+Y; W135+Y; A+B+C; A+B+W135; A+B+Y; A+C+W135; A+C+Y;

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A+W135+Y; B+C+W135; B+C+Y; C+W135+Y; A+B+C+W135; A+B+C+Y; B+C+W135+Y; and A+B+C+W135+Y.

Because sub capsular antigens are shared between serogroups then OMVs (and OMV mixtures) can protect against more than just the serogroup from which they are prepared. For example, the sub capsular antigens from serogroup A and W135 strains seen in sub-saharan Africa are shared with serogroup C and Y strains seen elsewhere in the world. Thus the invention provides the use of OMVs from a meningococcal strain in a first serogroup for protecting against one or more meningococcal strains in a second serogroup, wherein said first and second serogroups are different. The strains

preferably share sub-capsular antigens, and may have the same subtype, serosubtype and/or immunotype, even though they have different serogroups. A mixture of OMVs from serogroups A and W135 is preferred, as is a mixture of OMVs from serogroups C and Y.

Experience with MeNZB™ has also suggested to the inventors that a mixture of OMVs from the strains used for the New Zealand OMVs, the Norway OMVs and the Cuban OMVs would be usefully efficacious. Thus the invention provides a composition comprising outer membrane vesicles from two or three of: (i) a serosubtype P1.7b,4 meningococcus; (ii) a serosubtype P1.7,16 meningococcus; and (iii) a serosubtype P1.9,15 meningococcus. The different OMVs are preferably in admixture but, alternatively, they may be in separate containers within a kit.

In combining OMVs from different serosubtypes, the inventors have found that doses for individual serosubtypes can be reduced without loss of efficacy. Whereas VA-MENGOC-B™ contains 50µg of OMVs (0.5ml volume), HexaMen™ includes around 1mg OMVs (0.3ml volume), and both MenBVac™ and MeNZB™ contain 25µg OMVs (0.5ml volume), measured as total protein, the inventors have found that the dose of individual OMVs can be reduced when a mixture is used without loss of individual efficacy. Thus the invention provides a composition comprising outer membrane vesicles from a first meningococcal serosubtype and a second meningococcal serosubtype, wherein the concentration of OMVs from the first serosubtype is less than 45µg/ml and the concentration of OMVs from the second serosubtype is less than 45µg/ml. The invention also provides a composition comprising outer membrane vesicles from at least two meningococcal serosubtypes, wherein the combined concentration of OMVs is less than 90µg/ml. The invention also provides a composition comprising outer membrane vesicles from n different meningococcal serosubtypes, wherein the concentration of OMVs from each of the n serosubtypes is less than 45µg/ml (*i.e.* a total OMV dose of less than $45n$ µg/ml). The value of n may be 1, 2, 3, 4, 5, 6, *etc.*

The invention also provides a kit comprising OMVs prepared from n different serosubtypes. The vesicles can be kept and stored separately in the kit until they are required to be used together *e.g.* as an admixture, or for simultaneous separate or sequential use. Similarly, the invention provides a process comprising: preparing n sets of OMVs, one from each of n different serosubtypes; and combining the n sets of vesicles. The different sets can be combined into a kit or into an admixture.

The invention also provides a composition comprising OMVs prepared from a serogroup B meningococcal strain having a P1.7b,4 serosubtype, wherein the concentration of OMVs in the composition is about 50µg/ml. The composition preferably includes an aluminium hydroxide adjuvant and a histidine buffer. The composition may be given in a dose volume of about 0.5ml.

The vesicles

The invention is based on outer membrane vesicles (OMVs) prepared from *Neisseria meningitidis*. The term "OMV" includes any proteoliposomic vesicle obtained by disrupting a bacterial outer membrane to form vesicles of the outer membrane that include protein components of the outer

membrane. OMVs are prepared artificially from bacteria (*e.g.* by detergent treatment, or by non-detergent means [29]). The term also encompasses blebs, microvesicles (MVs [12]) and 'native OMVs' ('NOMVs' [13]), which are naturally-occurring membrane vesicles that form spontaneously during bacterial growth and are released into culture medium. MVs can be obtained by culturing *Neisseria* in broth culture medium, separating whole cells from the smaller MVs in the broth culture medium (*e.g.* by filtration or by low-speed centrifugation to pellet only the cells and not the smaller vesicles), and then collecting the MVs from the cell-depleted medium (*e.g.* by filtration, by differential precipitation or aggregation of MVs, by high-speed centrifugation to pellet the MVs). Strains for use in production of MVs can generally be selected on the basis of the amount of MVs produced in culture *e.g.* refs. 14 & 15 describe *Neisseria* with high MV production.

OMVs can be prepared in various ways. Methods for obtaining suitable preparations are disclosed in, for instance, the references cited herein. Techniques for forming OMVs include treating bacteria with a bile acid salt detergent (*e.g.* salts of lithocholic acid, chenodeoxycholic acid, ursodeoxycholic acid, deoxycholic acid, cholic acid, ursocholic acid, *etc.*, with sodium deoxycholate [16 & 17] being preferred for treating *Neisseria*) at a pH sufficiently high not to precipitate the detergent [6]. Other techniques may be performed substantially in the absence of detergent [29] using techniques such as sonication, homogenisation, microfluidisation, cavitation, osmotic shock, grinding, French press, blending, *etc.*

A preferred method for OMV preparation involves ultrafiltration [18] instead of high speed centrifugation on crude OMVs. This allows much larger amounts of OMV-containing supernatant to be processed in a much shorter time (typically >15 litres in 4 hours, compared to <1.5 litres in 10 hours), and avoids the need to redisperse OMVs after centrifugation. Ultracentrifugation allows large quantities of OMVs to be prepared much more easily, and permits the rapid production of OMVs from a strain of choice, for use in vaccine preparation.

Meningococcal strains used for vaccine preparation

Identifying the serosubtype of a meningococcal strain of interest can be achieved using standard techniques, based on the class I porin outer membrane protein (PorA). Once a serosubtype has been determined then it is routine to search for other known strains that share the same serosubtype. The other strains may share serogroup and/or serotype (PorB) with the first strain, but this will not necessarily be the case. In general, however, it is preferred to match both serogroup and serosubtype.

Meningococcal strains used according to the invention will generally be in one of the following serogroups: A, B, C, W135, or Y.

Meningococcal strains used according to the invention will generally not be strains that express multiple serosubtypes (*i.e.* multiple PorA alleles). Thus preferred bacteria for use with the invention will express a single PorA sequence *i.e.* they will be of a single serosubtype.

It is also possible to use strains in which PorA has been down-regulated *e.g.* in which the amount of PorA has been reduced by at least 20% (*e.g.* $\geq 30\%$, $\geq 40\%$, $\geq 50\%$, $\geq 60\%$, $\geq 70\%$, $\geq 80\%$, $\geq 90\%$, $\geq 95\%$, *etc.*), or even knocked out, relative to wild-type levels (*e.g.* relative to strain H44/76, as disclosed in reference 11).

- 5 Meningococci used according to the invention may be of any serotype (*e.g.* 1, 2a, 2b, 4, 14, 15, 16, *etc.*) and/or of any immunotype (*e.g.* L1; L3,3,7; L10; *etc.*). The meningococci may be from any suitable lineage, including hyperinvasive and hypervirulent lineages *e.g.* any of the following seven hypervirulent lineages: subgroup I; subgroup III; subgroup IV-1; ET-5 complex; ET-37 complex; A4 cluster; lineage 3. These lineages have been defined by multilocus enzyme electrophoresis (MLEE),
 10 but multilocus sequence typing (MLST) has also been used to classify meningococci [ref. 19] *e.g.* the ET-37 complex is the ST-11 complex by MLST, the ET-5 complex is ST-32 (ET-5), lineage 3 is ST-41/44, *etc.*

Meningococci may have one or more knockout mutations of gene(s). To reduce pyrogenic activity, for instance, the bacterium should have low endotoxin (LPS) levels, and this can be achieved by
 15 knockout of enzymes involved in LPS biosynthesis. Suitable mutant bacteria are already known *e.g.* mutant *Neisseria* [20,21] and mutant *Helicobacter* [22]. Processes for preparing LPS-depleted outer membranes from Gram-negative bacteria are disclosed in reference 23.

As well as down-regulating expression of specific proteins, the bacterium may over-express (relative to the corresponding wild-type strain) immunogens such as NspA, protein 287 [8], protein 741 [30],
 20 TbpA [7], TbpB [7], superoxide dismutase [7], *etc.*

As well as having knockouts of particular endogenous genes, the bacterium may express one or more genes that are not endogenous. For example, the invention may use a recombinant strain that expresses new genes relative to the corresponding wild-type strain. Expression of non-endogenous genes in this way can be achieved by various techniques *e.g.* chromosomal insertion (as used for
 25 introducing multiple PorA genes [24]), knockin mutations, expression from extra-chromosomal vectors (*e.g.* from plasmids), *etc.*

The bacterium may also include one or more of the knockout and/or over-expression mutations disclosed in references 25 to 30. Preferred genes for down-regulation and/or knockout include: (a) Cps, CtrA, CtrB, CtrC, CtrD, FrpB, GalE, HtrB/MsbB, LbpA, LbpB, LpxK, Opa, Opc, PilC, PorB,
 30 SiaA, SiaB, SiaC, SiaD, TbpA, and/or TbpB [25]; (b) CtrA, CtrB, CtrC, CtrD, FrpB, GalE, HtrB/MsbB, LbpA, LbpB, LpxK, Opa, Opc, PhoP, PilC, PmrE, PmrF, SiaA, SiaB, SiaC, SiaD, TbpA, and/or TbpB [26]; (c) ExbB, ExbD, rmpM, CtrA, CtrB, CtrD, GalE, LbpA, LpbB, Opa, Opc, PilC, PorB, SiaA, SiaB, SiaC, SiaD, TbpA, and/or TbpB [27]; and (d) CtrA, CtrB, CtrD, FrpB, OpA, OpC, PilC, PorB, SiaD, SynA, SynB, and/or SynC [28].

35 As well as combining OMVs based on different serosubtypes, combinations may be made according to other criteria. Example criteria include: serotype (PorB, class 2 or 3 OMP); immunotype

(lipopolysaccharide or lipooligosaccharide); geographical origin of the strains; local prevalence of clinical strains; hypervirulent lineage *e.g.* two or more of subgroups I, III and IV-1, ET-5 complex, ET-37 complex, A4 cluster and lineage 3; multilocus sequence type (MLST) [19]; more than one of the three different NMB1870 variants [31].

5 *OMV dosing*

Existing meningococcal OMV vaccines offer pharmaceutical, posological and formulation guidance for performing the invention. For example, VA-MENGOC-BC™ is an injectable suspension in 0.5ml that contains 50µg OMV from strain Cu-385-83 and 50µg serogroup C capsular polysaccharide, absorbed to 2mg of an aluminium hydroxide gel, plus 0.01% thiomersal and phosphate buffer. MeNZB™ is also a 0.5ml suspension, and contains 25µg OMV from strain NZ98/254 adsorbed on 1.65mg of an aluminium hydroxide adjuvant, with a histidine buffer and sodium chloride. MenBvac is similar to MeNZB™, but is prepared from strain 44/76.

The concentration of OMVs for each subtype will be high enough to provide protective immunity after administration to a patient. The concentration of OMVs in compositions of the invention will generally be between 10 and 500 µg/ml, preferably between 25 and 200µg/ml, and more preferably about 50µg/ml or about 100µg/ml (expressed in terms of total protein in the OMVs).

Where a composition include OMVs from more than one meningococcal serosubtype, however, the inventors have found that doses for individual serosubtypes can be reduced without loss of efficacy. In particular, the dose of the New Zealand and Norwegian subtypes can be halved from 25µg to 12.5µg in a 0.5ml dose without loss of immunogenicity. Thus a composition of the invention with outer membrane vesicles from more than one meningococcal subtype can include less than the 100µg/ml that would *a priori* be expected based on simple mixing of MenBvac™ and MeNZB™, and less than the 150µg/ml that would *a priori* be expected based on simple mixing of VA-MENGOC-BC™ with either MenBvac™ or MeNZB™. Thus such compositions of the invention will have a combined OMV concentration of no more than 90µg/ml (*e.g.* no more than 80µg/ml, 70µg/ml, 60µg/ml, 50µg/ml, 40µg/ml, 30µg/ml, or even lower).

More generally, where a composition includes outer membrane vesicles from *n* different meningococcal subtypes, the concentration of OMVs from each of the subtype is less than 45µg/ml (*e.g.* less than 40µg/ml, 35µg/ml, 30µg/ml, 25µg/ml, 20µg/ml, or even lower). A concentration of about 25µg/ml is preferred.

Where a composition includes outer membrane vesicles from *n* different meningococcal subtypes, the amount of OMVs for each subtype is preferably within $\pm 10\%$ of each other *i.e.* the composition includes substantially equal masses of each OMV. In some circumstances, however, the amount of one subtype may be about *x* times greater than the amount of another subtype, where *x* is 2, 3 or 4 *e.g.* the composition could include a double dose of one subtype relative to other subtype(s) in the composition.

Pharmaceutical compositions containing OMVs

Compositions of the invention may be pharmaceutical compositions that include a pharmaceutically acceptable carrier. Such compositions can be prepared using a process comprising the step of admixing OMVs with the pharmaceutically acceptable carrier.

- 5 Typical 'pharmaceutically acceptable carriers' 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, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The
- 10 vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, sucrose, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier (*e.g.* based on water for injection). A thorough discussion of pharmaceutically acceptable excipients is available in reference 32.
- 15 Compositions of the invention will typically be in aqueous form (*e.g.* solutions or suspensions) rather than in a dried form (*e.g.* lyophilised). Aqueous compositions are also suitable for reconstituting other vaccines from a lyophilised form (*e.g.* a lyophilised Hib conjugate vaccine, a lyophilised meningococcal conjugate vaccine, *etc.*). Where a composition of the invention is to be used for such extemporaneous reconstitution, the invention provides a kit, which may comprise two vials, or may
- 20 comprise one ready-filled syringe and one vial, with the aqueous contents of the syringe being used to reactivate the dried contents of the vial prior to injection.

Compositions of the invention may be presented in vials, or they may be presented in ready-filled syringes. The syringes may be supplied with or without needles. Compositions may be packaged in unit dose form or in multiple dose form. A syringe will generally include a single dose of the

25 composition, whereas a vial may include a single dose or multiple doses. For multiple dose forms, therefore, vials are preferred to pre-filled syringes.

Effective dosage volumes can be routinely established, but a typical human dose of the composition has a volume of about 0.5ml *e.g.* for intramuscular injection. The RIVM OMV-based vaccine was administered in a 0.5ml volume [33] by intramuscular injection to the thigh or upper arm. MeNZB™

30 is administered in a 0.5ml by intramuscular injection to the anterolateral thigh or the deltoid region of the arm. Similar doses may be used for other delivery routes *e.g.* an intranasal OMV-based vaccine for atomisation may have a volume of about 100µl or about 130µl per spray [13], with four sprays administered to give a total dose of about 0.5ml.

The pH of the composition is preferably between 6 and 8, and more preferably between 6.5 and 7.5

35 (*e.g.* about 7). The pH of the RIVM OMV-based vaccine is 7.4 [34], and a pH <7.5 is preferred for

compositions of the invention. Stable pH may be maintained by the use of a buffer *e.g.* a Tris buffer, a phosphate buffer, or a histidine buffer. Compositions of the invention will generally include a buffer. If a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [35] *e.g.* at between 1-10mM, preferably about 5mM. The RIVM OMV-based vaccine
5 maintains pH by using a 10mM Tris/HCl buffer. The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions of the invention are immunogenic, and are more preferably vaccine compositions. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Immunogenic compositions
10 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,
15 *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. The antigen content of compositions of the invention will generally be expressed in terms of the amount of protein per dose. A dose of about 0.9 mg
20 protein per ml is typical for OMV-based intranasal vaccines [13].

Meningococci 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. 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
25 pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as spray, drops, gel or powder [*e.g.* refs 36 & 37]. Injectables for intramuscular administration are typical.

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

30 Compositions of the invention may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

Compositions of the invention may include residual detergent (*e.g.* deoxycholate) from OMV preparation. The amount of residual detergent is preferably less than 0.4µg (more preferably less than 0.2µg) for every µg of protein.

35 Compositions of the invention may include LPS from meningococcus. The amount of LPS is preferably less than 0.12µg (more preferably less than 0.05µg) for every µg of protein.

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. The concentration of sodium chloride is preferably about 9 mg/ml.

5 Compositions of the invention will generally be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include one or more adjuvants, and the invention provides a process for preparing a composition of the invention, comprising the step of admixing vesicles of the invention with an adjuvant *e.g.* in a pharmaceutically acceptable carrier. Suitable adjuvants include, but are not limited to:

A. Mineral-containing compositions

10 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. 38], 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
15 mineral containing compositions may also be formulated as a particle of metal salt [39].

A typical aluminium phosphate adjuvant is amorphous aluminium hydroxyphosphate with PO₄/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al³⁺/ml. Adsorption with a low dose of aluminium phosphate may be used *e.g.* between 50 and 100µg Al³⁺ per dose. Where an aluminium phosphate it used and it is desired not to adsorb an antigen to the adjuvant, this is favoured by
20 including free phosphate ions in solution (*e.g.* by the use of a phosphate buffer).

The RIVM vaccine was tested with adsorption to either an aluminium phosphate or an aluminium hydroxide adjuvant, and the aluminium phosphate adjuvant was found to give superior results [34]. The MeNZBTM, MenBvacTM and VA-MENINGOC-BCTM products all include an aluminium hydroxide adjuvant.

25 A typical dose of aluminium adjuvant is about 3.3 mg/ml (expressed as Al³⁺ concentration).

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 38; see also ref. 40] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's
30 adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 38]

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*
35 Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap

root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

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
5 QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 41. Saponin formulations may also comprise a sterol, such as cholesterol [42].

Combinations of saponins and cholesterol can be used to form unique particles called immunostimulating complexes (ISCOMs) [chapter 23 of ref. 38]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be
10 used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA and QHC. ISCOMs are further described in refs. 42-44. Optionally, the ISCOMS may be devoid of extra detergent [45].

A review of the development of saponin based adjuvants can be found in refs. 46 & 47.

D. Virosomes and virus-like particles

Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These
15 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),
20 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. 48-53. Virosomes are discussed further in, for example, ref. 54

E. Bacterial or microbial derivatives

25 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.

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
30 chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 55. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 μ m membrane [55]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [56,57].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is
35 described for example in refs. 58 & 59.

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.

5 The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 60, 61 and 62 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. 63-68.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [69]. The
10 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 a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 70-72. Preferably, the CpG is a CpG-A ODN.

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
15 "immunomers". See, for example, refs. 69 & 73-75.

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. 76 and as parenteral adjuvants in ref. 77. The toxin or toxoid is preferably in the
20 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. 78-85. Numerical reference for amino acid substitutions is preferably based on the alignments of the
25 A and B subunits of ADP-ribosylating toxins set forth in ref. 86, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

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 [87], *etc.*) [88], interferons (*e.g.* interferon-
30 ?), macrophage colony stimulating factor, and tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [89] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone,
35 polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [90].

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150µm in diameter, more preferably ~200nm to ~30µm in diameter, and most preferably ~500nm to ~10µm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(a-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. 38)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 91-93.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [94]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [95] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [96]. 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)

PCPP formulations are described, for example, in refs. 97 and 98.

L. Muramyl peptides

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.

Examples of imidazoquinolone compounds suitable for use as adjuvants in the invention include Imiquimod and its homologues (*e.g.* "Resiquimod 3M"), described further in refs. 99 and 100.

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 [101]; (2) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) [102]; (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) [103]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [104]; (6) SAF, containing 10% squalene, 0.4% Tween 80TM, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) RibiTM 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).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 38.

5 The use of aluminium salt adjuvants is particularly preferred, and antigens are generally adsorbed to such salts. 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. In general, however, it is preferred to use only a single salt *e.g.* a hydroxide or a phosphate, but not both. Not all vesicles need to be adsorbed *i.e.* some or all can be free in solution.

Methods of treatment

10 The invention also provides a method for raising an immune response in a mammal, comprising administering a pharmaceutical composition of the invention to the mammal. The immune response is preferably protective and preferably involves antibodies. The method may raise a booster response in a patient that has already been primed against *N.meningitidis*. Subcutaneous and intranasal prime/boost regimes for OMVs are disclosed in ref. 105.

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

The invention also provides OMV compositions and mixtures of the invention for use as a
20 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.

The invention also provides the use of OMV compositions and mixtures of the invention in the manufacture of a medicament for raising an immune response in a mammal.

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

One way of checking efficacy of therapeutic treatment involves monitoring Neisserial infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against OMV antigens after administration of the composition. Immunogenicity of compositions of the invention can be determined by administering
30 them to test subjects (*e.g.* children 12-16 months age, or animal models [106]) and then determining standard parameters including serum bactericidal antibodies (SBA) and ELISA titres (GMT). 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
35 administered, more than one post-administration determination may be made.

Preferred compositions of the invention can confer an antibody titre in a patient that is superior to the criterion for seroprotection 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%.

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.

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. The OMV-based RIVM vaccine was tested using a 3- or 4-dose primary schedule, with vaccination at 0, 2 & 8 or 0, 1, 2 & 8 months. MeNZB™ is administered as three doses at six week intervals.

As described above, the invention may involve administration of vesicles from more than one serosubtype of *N.meningitidis*, either separately or in admixture.

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

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 166]. 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. MeNZB™ elicits a 4-fold rise in SBA 4-6 weeks after administration of the third dose.

By mixing OMVs for different serosubtypes, compositions of the invention may induce bactericidal antibody responses against more than one hypervirulent lineage of meningococcus. In particular, they can preferably 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. This does not necessarily mean that the composition can induce bactericidal antibodies against each and

every strain of meningococcus within these hypervirulent lineages *e.g.* rather, for any given group of four or more strains of 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.* 2¹⁰, 2¹¹, 2¹², 2¹³, 2¹⁴, 2¹⁵, 2¹⁶, 2¹⁷, 2¹⁸ or higher, preferably at least 2¹⁴) *e.g.* the serum is able to kill at least 50% of test bacteria of a particular strain when diluted 1/1024, as described in reference 166.

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.

Strains 961-5945 and G2136 are both *Neisseria* MLST reference strains [ids 638 & 1002 in ref. 107]. Strain MC58 is widely available (*e.g.* ATCC BAA-335) and was the strain sequenced in reference 108. Strain 44/76 has been widely used and characterised (*e.g.* ref. 109) and is one of the *Neisseria* MLST reference strains [id 237 in ref. 107; row 32 of Table 2 in ref. 19]. Strain 394/98 was originally isolated in New Zealand in 1998, and there have been several published studies using this strain (*e.g.* refs. 110 & 111). Strain BZ198 is another MLST reference strain [id 409 in ref. 107; row 41 of Table 2 in ref. 19].

Further antigenic components

As well as containing OMVs, compositions of the invention may include further non-vesicular antigens. For example, the composition may comprise one or more of the following further antigens:

- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 112 from serogroup C or the oligosaccharides of ref. 113. The VA-MENINGOC-BC™ product contains serogroup C polysaccharide.
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* refs. 114-116; ch.22 & 23 of ref. 123].
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 117,118; chapter 15 of ref. 123].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 118,119; chapter 16 of ref. 123].
- an antigen from hepatitis C virus [*e.g.* 120].
- 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. 121 & 122; chapter 21 of ref. 123].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 13 of ref. 123].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 27 of ref. 123].

- a saccharide antigen from *Haemophilus influenzae* B [e.g. chapter 14 of ref. 123]
- an antigen from *N.gonorrhoeae* [e.g. ref. 124].
- an antigen from *Chlamydia pneumoniae* [e.g. 125-131].
- an antigen from *Chlamydia trachomatis* [e.g. 132].
- 5 – an antigen from *Porphyromonas gingivalis* [e.g. 133].
- polio antigen(s) [e.g. 134, 135; chapter 24 of ref. 123] such as IPV.
- rabies antigen(s) [e.g. 136] such as lyophilised inactivated virus [e.g. 137, RabAvert™].
- measles, mumps and/or rubella antigens [e.g. chapters 19, 20 and 26 of ref. 123].
- influenza antigen(s) [e.g. chapters 17 & 18 of ref. 123], such as the haemagglutinin and/or
- 10 neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [e.g. 138].
- a protein antigen from *Streptococcus agalactiae* (group B streptococcus) [e.g. 139, 140].
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [e.g. 140, 141, 142].

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier in order to
 15 enhance immunogenicity. Conjugation of *H.influenzae* B, meningococcal and pneumococcal saccharide antigens is well known.

Toxic protein antigens may be detoxified where necessary (e.g. detoxification of pertussis toxin by chemical and/or genetic means [122]).

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

Saccharide antigens are preferably in the form of conjugates. Preferred carrier proteins for conjugates are bacterial toxins or toxoids, such as diphtheria toxoid or tetanus toxoid. The CRM197 mutant of
 25 diphtheria toxin [143-145] is a particularly preferred carrier for, as is a diphtheria toxoid. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [146], synthetic peptides [147,148], heat shock proteins [149,150], pertussis proteins [151,152], cytokines [153], lymphokines [153], hormones [153], growth factors [153], artificial proteins comprising multiple human CD4⁺ T cell epitopes from various pathogen-derived antigens [154], protein D from *H.influenzae* [155,156],
 30 pneumococcal surface protein PspA [157], pneumolysin [158], iron-uptake proteins [159], toxin A or B from *C.difficile* [160], 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 protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used. 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.

Preferred compositions include meningococcal OMVs as described above, plus a conjugated capsular saccharide from one or more (*i.e.* 1, 2, 3 or 4) of meningococcal serogroups A, C, W135 and Y. Where the OMVs are from serogroup B then this approach allows the following serogroups to be covered: B+A; B+C; B+W135; B+Y; B+C+W135; B+C+Y; B+W135+Y; B+A+C+W135; B+A+C+Y; B+A+W135+Y; B+C+W135+Y; and B+A+C+W135+Y. Two preferred combinations use serogroup B OMVs plus conjugate antigens from either serogroups A+W135+Y or serogroups A+C+W135+Y. In general, it is possible to cover all five of serogroups A, B, C, W135 and Y by choosing OMVs for x serogroup(s) and conjugated saccharides for the remaining $5-x$ serogroups.

Specific meningococcal protein antigens (preferably from serogroup B) may also be added to supplement the OMV compositions. In particular, a protein antigen such as disclosed in refs. 30 & 161 to 169 may be added. A small number of defined antigens may be added (a mixture of 10 or fewer (*e.g.* 9, 8, 7, 6, 5, 4, 3, 2) purified antigens). Preferred additional immunogenic polypeptides for use with the invention are those disclosed in reference 169: (1) a 'NadA' protein; (2) a '741' protein; (3) a '936' protein; (4) a '953' protein; and (5) a '287' protein. Other possible supplementing meningococcal antigens include transferrin binding proteins (*e.g.* TbpA and TbpB) and/or Cu,Zn-superoxide dismutase [7]. Other possible supplementing meningococcal antigens include proteins comprising one of the following amino acid sequences: SEQ ID NO:650 from ref. 161; SEQ ID NO:878 from ref. 161; SEQ ID NO:884 from ref. 161; SEQ ID NO:4 from ref. 162; SEQ ID NO:598 from ref. 163; SEQ ID NO:818 from ref. 163; SEQ ID NO:864 from ref. 163; SEQ ID NO:866 from ref. 163; SEQ ID NO:1196 from ref. 163; SEQ ID NO:1272 from ref. 163; SEQ ID NO:1274 from ref. 163; SEQ ID NO:1640 from ref. 163; SEQ ID NO:1788 from ref. 163; SEQ ID NO:2288 from ref. 163; SEQ ID NO:2466 from ref. 163; SEQ ID NO:2554 from ref. 163; SEQ ID NO:2576 from ref. 163; SEQ ID NO:2606 from ref. 163; SEQ ID NO:2608 from ref. 163; SEQ ID NO:2616 from ref. 163; SEQ ID NO:2668 from ref. 163; SEQ ID NO:2780 from ref. 163; SEQ ID NO:2932 from ref. 163; SEQ ID NO:2958 from ref. 163; SEQ ID NO:2970 from ref. 163; SEQ ID NO:2988 from ref. 163, 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 (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). Preferred fragments 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. The meningococcal antigens transferrin binding protein and/or Hsf protein may also be added [170].

Supplementation of the OMVs by defined meningococcal antigens in this way is particularly useful where the OMVs are from a serosubtype P1.7b,4 meningococcus or a serosubtype P1.7,16 meningococcus. Supplementation of a mixture of OMVs from both these serosubtypes is preferred.

Specific serosubtypes

- 5 The invention provides a composition comprising OMVs prepared from a meningococcus having one of the following subtypes: P1.2; P1.2,5; P1.4; P1.5; P1.5,2; P1.5,c; P1.5c,10; P1.7,16; P1.7,16b; P1.7h,4; P1.9; P1.15; P1.9,15; P1.12,13; P1.13; P1.14; P1.21,16; P1.22,14

The meningococcus is preferably in serogroup B.

These OMVs are suitable for use with the invention, as described above.

10 ***OMV administration regime***

The invention provides a method for administering a meningococcal OMV vaccine to a patient, wherein a first dose is given at time zero, a second and a third dose are given over the next two months, and a fourth dose is given between 11 and 13 months after time zero.

- 15 The invention also provides a method for administering meningococcal OMV vaccines to a patient, wherein a first dose is given at time zero, a second and a third dose are given over the next two months, and a fourth dose is given between 11 and 13 months after time zero, and wherein (a) the first, second and third doses comprise OMVs with the same serosubtype as each other, and (b) the fourth dose comprises OMVs with a different serosubtype from the first three doses. The fourth dose may contain OMVs only with a different serosubtype from the first three doses, or it may contain two
20 types of OMV, one with a different serosubtype from the first three doses and one with the same subtype.

The first, second and third doses are preferably given at intervals of between 6 and 8 weeks. The fourth dose is preferably given about 1 year after time zero.

The patient preferably receives the same quantity of vaccine at each of the four doses.

- 25 The OMVs are preferably serosubtype P1.7b,4 and/or P1.7,16.

The invention also provides a method for administering a meningococcal vaccine to a patient, wherein: (a) the vaccine comprises meningococcal OMVs having a first serosubtype; (b) the patient has previously received a different OMV vaccine having a second serosubtype, with the first dose of the different OMV vaccine was given more than 11 months before this method.

- 30 The invention also the use of meningococcal OMVs having a first serosubtype in the manufacture of a medicament for immunising against meningococcal meningitis, wherein the medicament is for administration to a patient that has been pre-immunised with OMVs having a second serosubtype.

OMV administration may also follow immunisation with a meningococcal conjugate vaccine. Thus the invention provides the use of meningococcal OMVs from a first meningococcal serogroup in the manufacture of a medicament for immunising against at least meningococcal meningitis, wherein the medicament is for administration to a patient that has been pre-immunised with a conjugated capsular
 5 saccharide from a second meningococcal serosubtype. Similarly, it provides a method for administering a meningococcal vaccine to a patient, wherein: (a) the vaccine comprises meningococcal OMVs having a first serogroup; (b) the patient has previously received a conjugated capsular saccharide from a second meningococcal serogroup.

The pre-immunisation may have taken place more than 6 months before the OMVs are administered
 10 (*e.g.* more than 11 months). Thus, for instance, a patient may receive conjugated saccharides at time zero, and then OMVs 11 months later.

The pre-immunisation with a meningococcal saccharide is preferably with at least serogroup C, but may be with more than one serogroup *e.g.* with both A+C, with A+C+Y, with A+C+W135+Y, *etc.*

The first serogroup is preferably serogroup B.

15 The OMVs may be administered at the same time as meningococcal conjugates *i.e.* the patient is receiving a further dose of meningococcal conjugate at the same time as receiving the OMVs.

The patient may or may not have been pre-immunised with OMVs from the first serogroup.

The patient may have been pre-immunised with a *H.influenzae* type b capsular saccharide conjugate.

The patient may have been pre-immunised with a diphtheria toxoid and a tetanus toxoid.

20 **General**

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

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

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
 25 omitted from the definition of the invention.

MODES FOR CARRYING OUT THE INVENTION

OMV dosage – single strain

Dosing studies for the McNZB™ product were performed on healthy adults. Adults received three
 30 doses of either 25µg or 50µg of OMV, given at 6 week intervals through a 25mm 23-gauge needle. A four-fold rise in SBA titre against the vaccine strain, measured 4 to 6 weeks after the third vaccination, was seen in 100% of patients receiving the 25µg dose but, surprisingly, was seen in only 87% of patients receiving the higher dose. The proportion of responders was also higher at the lower

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dosage after the second dose (87% vs. 78%). The lower dose was therefore selected for further use, thereby permitting stocks of vaccine to provide for immunisation of twice as many patients.

OMV dosage – multiple combined strains

OMVs prepared from Norwegian strain H44/76 have previously been described and administered to human patients in phase I, II and III clinical trials. They form the basis of the MenBvac™ product. Similarly, OMVs prepared from New Zealand strain HZ98/254 form the basis of the MeNZB™ product. Their safety and efficacy have been confirmed.

Both MeNZB™ and MenBvac™ include OMVs at a concentration of 50µg/ml (measured as amount of protein) in a 0.5ml dose. When testing a combination of the two OMVs in humans then, in order to maintain efficacy against the two different serosubtypes, the most direct comparison would be to keep the concentration of each OMV at 50µg/ml. In contrast, the inventors chose to keep the total OMV dose the same as in the two monovalent products (50µg/ml) and instead to halve the amount of each OMV *i.e.* to use 25µg/ml of each serosubtype.

The combined vaccine is administered to patients who have previously received either MeNZB™ or MenBvac™. The combination is given 1 year after the initial dose of the monovalent OMVs.

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

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition comprising an admixture of outer membrane vesicles from (i) a serosubtype P1.7b,4 meningococcus, and (ii) a serosubtype P1.7,16 meningococcus.
2. The composition of claim 1 comprising an admixture of outer membrane vesicles from (i) a serosubtype P1.7b,4 meningococcus, (ii) a serosubtype P1.7,16 meningococcus and (iii) meningococcus and a serosubtype P1.19, 15 meningococcus.
3. The composition according to claim 1 or 2, wherein the concentration of OMVs for each serosubtype is between 10µg/ml and 500µg/ml.
4. The composition according to any one of claims 1 to 3, wherein the concentration of OMVs for serosubtype P1.7b,4 is about 50µg/ml.
5. The composition according to any one of claims 1 to 4, further comprising outer membrane vesicles from a meningococcus having one of the following subtypes: P1.2; P1.2,5; P1.4; P1.5; P1.5,2; P1.5,c; P1.5c,10; P1.7,16b; P1.7h,4; P1.9; P1.15; P1.12,13; P1.13; P1.14; P1.21,16; and P1.22,14.
6. The composition according to any one of claims 1 to 5, further comprising outer membrane vesicles from 2 or 3 different meningococcal NMB 1870 variants.
7. The composition according to any one of claims 1 to 6, further comprising outer membrane vesicles from a meningococcus having a knockout of one or more enzymes involved in LPS biosynthesis.
8. The composition according to any one of claims 1 to 7, further comprising outer membrane vesicles from a meningococcus that over-expresses an immunogen selected from the group consisting of: NspA, protein 287, protein 741, TbpA, TbpB and superoxide dismutase.
9. The composition according to any one of claims 1 to 8, further comprising outer membrane vesicles from a meningococcal strain in a hyperinvasive or hypervirulent lineage.
10. The composition according to claim 9, wherein the hypervirulent lineage is selected from the group consisting of: subgroup I; subgroup III; subgroup TV 1; ET 5 complex; ET 37 complex; A4 cluster; and lineage 3.

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11. The composition according to any one of claims 1 to 10, further comprising conjugated capsular saccharide from one or more of meningococcal serogroups A, C, W135 and Y.
12. The composition according to any one of claims 1 to 11, further comprising conjugated capsular saccharide from two or more of meningococcal serogroups A, C, W135 and Y.
13. The composition according to any one of claims 1 to 12, comprising less than 0.4µg of deoxycholate for every µg of protein.
14. The composition according to any one of claims 1 to 13, comprising less than 0.12µg of meningococcal LPS for every µg of protein.
15. The composition according to any one of claims 1 to 14, wherein the composition comprises an aluminium hydroxide adjuvant.
16. The composition according to any one of claims 1 to 15, wherein the composition comprises an aluminium hydroxide adjuvant and a histidine buffer.
17. The composition according to any one of claims 1 to 16, comprising OMVs from serosubtype P1.7b,4 meningococcus at a concentration of about 25 µg/mL and/or OMVs from serosubtype P1.7,16 meningococcus at a concentration of about 25 µg/mL.
18. The composition according to any one of claims 1 to 17, wherein the total concentration of the admixture of OMVs is about 50 µg/mL.
19. A method of preparing a meningococcal outer membrane vesicle (OMV) vaccine, said method comprising admixing outer membrane vesicles from (i) a serosubtype P1.7b,4 meningococcus and (ii) a serosubtype P1.7,16 meningococcus.
20. The method of claim 19, comprising admixing outer membrane vesicles (i) a serosubtype P1.7b,4 meningococcus, (ii) a serosubtype P1.7,16 meningococcus and (iii) a serosubtype P1.19, 15 meningococcus.
21. The method according to claim 19 or claim 20, further comprising admixing outer membrane vesicles from a meningococcus having one of the following subtypes: P1.2; P1.2,5; P1.4; P1.5; P1.5,2; P1.5,c; P1.5c,10; P1.7,16b; P1.7h,4; P1.9; P1.15; P1.12,13; P1.13; P1.14; P1.21,16; and P1.22,14.

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22. The method according to any one of claims 19 to 21, further comprising admixing outer membrane vesicles from 2 or 3 different meningococcal NMB 1870 variants.
23. The method according to any one of claims 19 to 22, further comprising admixing outer membrane vesicles from a meningococcus having a knockout of one or more enzymes involved in LPS biosynthesis.
24. The method according to any one of claims 19 to 23, further comprising admixing outer membrane vesicles from a meningococcus that over-expresses an immunogen selected from the group consisting of: NspA, protein 287, protein 741, TbpA, TbpB and superoxide dismutase.
25. The method according to any one of claims 19 to 24, further comprising admixing outer membrane vesicles from a meningococcal strain in a hyperinvasive or hypervirulent lineage.
26. The method according to claim 25, wherein the hypervirulent lineage is selected from the group consisting of: subgroup I; subgroup III; subgroup TV 1; ET 5 complex; ET 37 complex; A4 cluster; and lineage 3.
27. The method according to any one of claims 19 to 26, further comprising admixing conjugated capsular saccharide from one or more of meningococcal serogroups A, C, W135 and Y.
28. The method according to any one of claims 19 to 27, further comprising admixing conjugated capsular saccharide from two or more of meningococcal serogroups A, C, W135 and Y.
29. The method according to any one of claims 19 to 28, further comprising admixing an aluminium hydroxide adjuvant with the outer membrane vesicles, optionally in a histidine buffer.
30. The method according to any one of claims 19 to 29, comprising admixing OMVs from a serosubtype P1.7b,4 meningococcus at a concentration of about 25 µg/mL and/or admixing OMVs from serosubtype P1.7,16 meningococcus at a concentration of about 25 µg/mL.
31. The method according to any one of claims 19 to 30, comprising admixing OMVs to a total concentration of about 50 µg/mL.
32. A composition when prepared by performing the method according to any one of claims 19 to 31.

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33. The composition according to claim 15 or 16 or according to claim 17 or 18 when appended to claim 15 or claim 15 or according to claim 32 when appended to any one of claims 29 to 31, wherein said composition is in a dose volume of about 0.5ml.

34. A method of preventing *meningococcal meningitis* infection in a subject or immunizing a subject against *meningococcal meningitis*, said method comprises administering to a subject the composition according to any one of claims 1 to 18, 32 or 33.

35. The method of claim 34, comprising administering to the subject at least four dosage units of the composition, optionally wherein the a dosage unit is administered at time zero, a second and third dosage units is each administered during a two month period after time zero, and a fourth unit dosage is administered between 11 and 13 months after time zero.

36. The method of claim 35, wherein the first, second and third dosage units comprise OMVs of the same serosubtype as each other, and the fourth dosage unit comprises OMVs of a different serosubtype from the first three doses.

37. Use of the composition according to any one of claims 1 to 18, 32 or 33 in immunization against *meningococcal meningitis* or preventing *meningococcal meningitis* infection in a subject.

38. Use of the composition according to any one of claims 1 to 18, 32 or 33 in the preparation of a medicament for use in immunization against *meningococcal meningitis* or preventing *meningococcal meningitis* infection in a subject.

39. A method of preventing *meningococcal meningitis* infection in a subject or immunizing a subject against *meningococcal meningitis* comprising administering to a subject of outer membrane vesicles from

- (i) a serosubtype P1.7b,4 meningococcus; and
- (ii) a serosubtype P1.7,16 meningococcus; and optionally
- (iii) a serosubtype P 1.19, 15 meningococcus,

wherein said subject that has been pre-immunized with a composition comprising outer membrane vesicles of a different serosubtype.

40. Use of outer membrane vesicles from

- (i) a serosubtype P1.7b,4 meningococcus; and
- (ii) a serosubtype P1.7,16 meningococcus; and optionally
- (iii) a serosubtype P 1.19, 15 meningococcus,

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in preventing *meningococcal meningitis* infection in a subject or immunizing a subject against *meningococcal meningitis*, wherein the subject has been pre-immunized with a composition comprising outer membrane vesicles of a different serosubtype.

5 41. Use of outer membrane vesicles from

(i) a serosubtype P1.7b,4 meningococcus; and

(ii) a serosubtype P1.7,16 meningococcus; and optionally

(iii) a serosubtype P 1.19, 15 meningococcus,

in the preparation of a medicament for preventing *meningococcal meningitis* infection in a subject or immunizing a subject, wherein said subject has been pre-immunized with a composition comprising outer membrane vesicles of a different serosubtype.

42. The method of claim 39 or the use of claim 40 or 41, wherein the subject has been pre-immunized more than 11 months beforehand.

43. A kit comprising a plurality of dosage units of the composition according to any one of claims 1 to 18, 32 or 33.

44. The kit according to claim 43, comprising four dosage units of the composition.

45. The kit according to claim 44, comprising four dosage units wherein the first, second and third dosage units comprise OMVs of the same serosubtype as each other, and the fourth dosage unit comprises OMVs of a different serosubtype from the first three doses.

46. The kit according to claim 44 or 45, packaged with instructions to use a first dose is given at time zero, a second dose and a third dose over the next two months, and a fourth dose between 11 and 13 months after time zero.

47. Use of the kit according to any one of claims 43 to 46 in preventing meningococcal meningitis infection or in immunization against meningococcal meningitis.

48. Use of the kit according to any one of claims 43 to 46 in the preparation of a medicament for preventing meningococcal meningitis infection or for immunization against meningococcal meningitis.

49. The composition according to any one of claims 1 to 18, 32 or 33, or the method according to any one of claims 19 to 31, 34 to 36, 39 or 42 or the use according to any one of claims 37, 38, 40, 41, 47 or 48 or the kit according to any one of claims 43 to 42 substantially as herein before described with reference to the Examples.

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Novartis Vaccines and Diagnostics, Inc.

By Patent Attorneys for applicant:
FB RICE